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METABOLITES PRODUCED BY *ALTERNARIA BRASSICAE*, THE BLACK SPOT PATHOGEN OF CANOLA.[1] PART 1, THE PHYTOTOXIC COMPONENTS

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ABSTRACT.—The metabolites produced in still culture by *Alternaria brassicae*, the phytopathogen responsible for the black spot disease of canola, have been investigated. A host specific phytotoxin responsible for symptom expression has been identified as the known cyclodepsipeptide destruxin B [**3**]. Two other cyclodepsipeptides, the known desmethyldestruxin B [**7**] and the previously unreported homodestruxin B [**4**], have also been isolated and identified.

Alternaria brassicae (Berkeley) Saccardo is the fungus responsible for the black spot disease in canola (rapeseed) plants. Canola plants are an important crop for oilseed production in western Canada. When plants are infected with the pathogen *A. brassicae*, there is a substantial reduction in the canola seed yield and the quality of the oil. The disease manifests itself most visibly on the leaves, where it appears as a thin black spot known as the necrotic center surrounded by a yellow halo or chlorotic zone. The pathogen attacks all parts of the plant, particularly the leaves, and it has been suggested that the chlorotic effect is induced by a phytotoxin (1).

A significant number of non-specific phytotoxins (2-6) showing a wide range of disease symptoms and diverse chemical properties have been isolated from culture filtrates of several phytopathogenic *Alternaria* species. They are all non-enzymatic compounds with low molecular weights, ranging from small peptides to simple phenols. Among the most important are alternariol methyl ether (7), which is a causative agent of necrosis on pear leaves and chlorosis on tobacco leaves; tenuazonic acid, a phytotoxic tetramic acid not unique to *Alternaria* fungi, known to produce necrotic lesions with or without chlorosis in plants (7); zinniol, a nonselective phytotoxin produced by several *Alternaria* species (8); and the cyclic tetrapeptide tentoxin [**1**] (9-12), produced by *Alternaria tenuis* and reported to cause strong chlorosis in cotyledons of some plant species.

Because very little chemical work has been reported on the toxic components of *A. brassicae*[2], we set out to isolate and identify these compounds. In this paper we report the isolation and identification of the phytotoxic metabolites of *A. brassicae*.

RESULTS AND DISCUSSION

The fungus was grown in liquid, still culture for 20 days on a medium containing 10% V-8 juice and 1% glucose. The mycelial mat was separated from the culture broth, air dried, and extracted with EtOAc. Removal of EtOAc afforded a crude mycelial extract. The culture broth was concentrated to about 1/10 of its original volume, then extracted with EtOAc. Removal of EtOAc gave the crude broth extract. Each of the extracts and the culture broth was tested for biological activity. The biological activity, i.e., the ability to induce chlorosis, was observed in the aqueous culture filtrates obtained from the fungal growth when these were tested on canola leaves using the leaf-spot bioassay. However, a problem was encountered with the bioassay of the organic ex-

[1]Presented at the 68th Annual Conference, Chemical Institute of Canada, Kingston, Ontario, June 1985.

[2]We thank J.P. Tewari, Professor of Plant Science, the University of Alberta, for drawing our attention to this problem and for providing cultures of *A. brassicae*.

tracts. This arose as a result of the poor solubility of the crude metabolites in H_2O. When the crude extracts were dissolved in organic solvents and the leaf-spot bioassay was attempted, no positive tests were obtained. The fact that the toxic components was present in the organic extract was demonstrated by the fact that the biological activity present in the aqueous culture filtrates was lost after extraction of the filtrate with EtOAc. Eventually a reliable, reproducible bioassay was developed, that was a modification of one reported for the isolation of the AM-toxins [**2**] (13-15) from *Alternaria mali* (apple pathotype of *Alternaria alternata*), the fungus reported to be the cause of alternaria blotch of apple (2-8). In this bioassay the test component is adsorbed on Si gel. The impregnated Si gel is placed on the canola leaf and wetted with a small amount of H_2O. The leaf is placed in a petri dish in a moist atmosphere at room temperature. In a positive test, chlorosis is observed with 48 h. Using this assay procedure it was possible to determine that strong biological activity is present in the organic extracts of the broth from *A. brassicae* grown on V-8 juice medium.

Purification of the active crude extract was initiated by suspension in a mixture of H_2O-MeOH (ca. 9:1, v/v), and successively partitioning the resulting suspension between hexane (low-polarity fraction), EtOAc (medium-polarity fraction), and *n*-BuOH (high-polarity fraction). Bioassay of the three fractions showed that the medium polarity fraction, which was the largest fraction, possessed the strongest chlorosis-inducing ability. The medium polarity fraction was separated by "dry flash column chromatography" (see Experimental) into 17 fractions. Each of these fractions was tested for biological activity. Fractions 10 and 11 gave positive results, both fractions showing the same major component by tlc. The active fractions were combined and purified further by dry flash column chromatography to yield two pure compounds which differed in the way they visualize on tlc. The most abundant crystalline component, A, is faintly visible, whereas the minor component, B, appears with a characteristic blue color when developed with phosphomolybdic acid followed by charring. The structure of B, which does not induce chlorosis, is presented in the accompanying paper.

Component A was purified by recrystallization from Skellysolve B/Me_2CO to give an optically active, crystalline component (mp 225-227°) that was found to have a molecular formula of $C_{30}H_{51}N_5O_7$ (M^+ 593) by hrms. The molecular weight of A was confirmed by fab ms where peaks at *m/z* 1188 [2(M+1)] and *m/z* 594 (M+1) are observed. The molecular formula of A suggested that it may be a polypeptide. Further evidence for the peptide nature of A was given by the similarity of its ir spectrum with that reported for cyclodepsipeptides (16) including the AM-toxins [**2**]. This characteristic spectrum displays N-H bands at 3360 cm^{-1} and 3290 cm^{-1}, and ester carbonyl absorption at 1730 cm^{-1}, amide I bands at 1670 cm^{-1} and 1630 cm^{-1}, and an amide II band at 1515 cm^{-1}.

The ^{1}H-nmr spectrum of compound A shows some striking similarities to that of the aforementioned tentoxin [**1**] (9-12). The similarities include the doublets at δ 8.19 and δ 7.17 corresponding to hydrogens on nitrogen and the singlets (each 3 H) at δ 3.20 and δ 2.73 corresponding to methyl groups attached to nitrogen. This accounts for four of the nitrogens of compound A. The fifth nitrogen must be present as a tertiary amide. The ^{13}C-nmr spectrum shows six carbonyl carbons between δ 169 and δ 174, in agreement with the presence of one ester and five amides.

Vigorous acid hydrolysis of compound A, followed by amino acid analysis, revealed the presence of β-alanine, isoleucine, and proline present in equimolar quantities (*N*-methylated amino acids are not identified by this method). Assuming that we are dealing with a cyclodepsipeptide, the presence of proline accounts for the remaining unsaturation implied by the molecular formula.

Methanolysis (K_2CO_3-MeOH) opened the lactone ring of compound A to give the

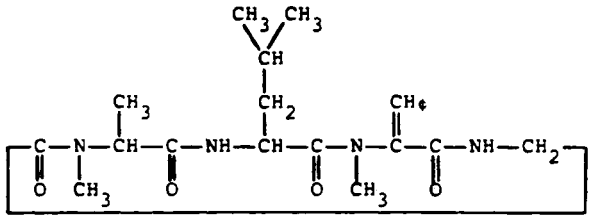
corresponding hydroxyester as an inseparable mixture of two diastereoisomers. The hreims of this ester, discussed below, suggested that compound A is the known cyclodepsipeptide destruxin B [3], first isolated from cultures of *Metarrhizium anisopliae*, a fungus which is pathogenic to silkworms (13-15). Comparison (ir, ^{1}H nmr, optical rotation, mmp) of compound A with an authentic sample of destruxin B [3] confirmed the identity. Destruxin B [3] shows very strong chlorosis inducing activity when tested on canola leaves.

The chromatographic fractions which afforded destruxin B contained a second, less polar component which showed similar charring behavior on visualization of the tlc plates. Purification by preparative tlc gave this component, which we have named homodestruxin B, in pure form. Homodestruxin B has the molecular formula $C_{31}H_{53}N_5O_7$, differing from that of destruxin B by having an additional methylene group.

The location of the extra methylene group in homodestruxin B [4] was determined by comparison of the mass spectrum of its methanolysis product **5** with that of the above mentioned methanolysis product of destruxin B [6] (Scheme 1). It has been shown that a prominent fragmentation pattern observed with the hydroxyesters derived from cyclodepsipeptides results from cleavage of peptide bonds, the cleavage originating at the carboxylate end (16) as illustrated in Scheme 1 for the destruxin B derivative **6**. The homodestruxin B derivative shows the same *m/z* 212 and 325 peaks, but the *m/z* 438 peak is replaced by one at *m/z* 452, revealing the *N*-methylvaline has been replaced by a higher homolog. The ^{1}H-nmr spectrum of homodestruxin B does not show the isopropyl signals present in the spectrum of destruxin B but rather shows two methyl triplets in place of the single one observed for **3**. A methine proton at δ 4.99, characteristic of hydrogens α to amide nitrogen, appears as a sharp doublet in the spectrum of homodestruxin B. These data are consistent with structure **4** for homodestruxin B in which an *N*-methylisoleucine replaces the *N*-methylvaline of destruxin B.

Desmethyldestruxin B [7], previously isolated as a metabolite of *M. anisopliae*, was the third cyclodepsipeptide isolated from the organic extracts of *A. brassicae* culture filtrates. It is the most polar of the three and was purified from a mixture containing destruxin B [3], homodestruxin B [4], and desmethyldestruxin B [7] by preparative tlc.

Desmethyldestruxin B has the molecular formula $C_{29}H_{49}N_5O_7$ (M^+579), indica-



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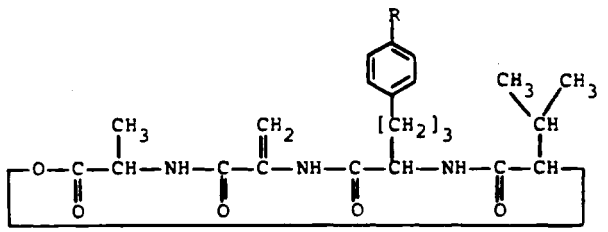
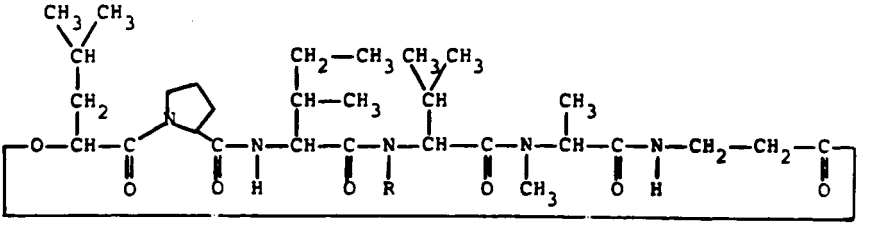
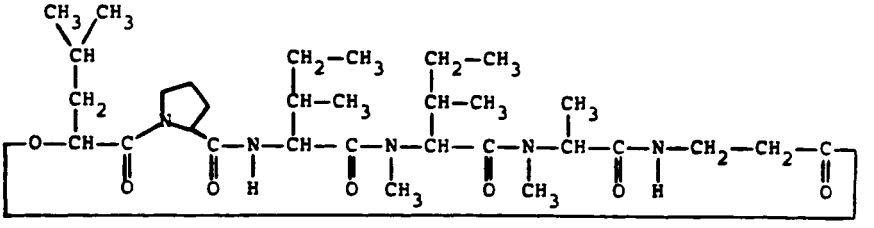




3 R=CH_3
7 R=H



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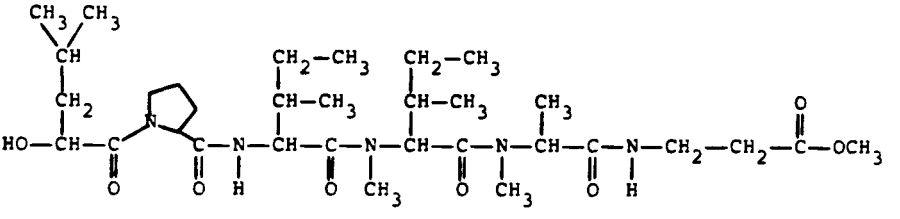

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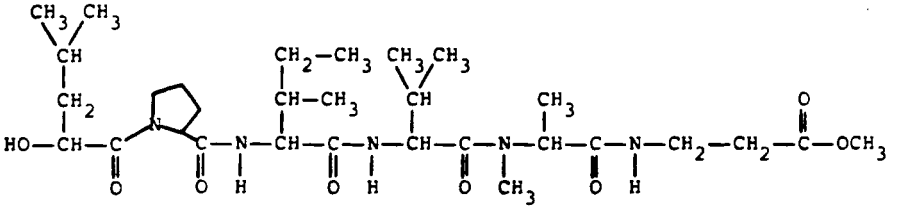

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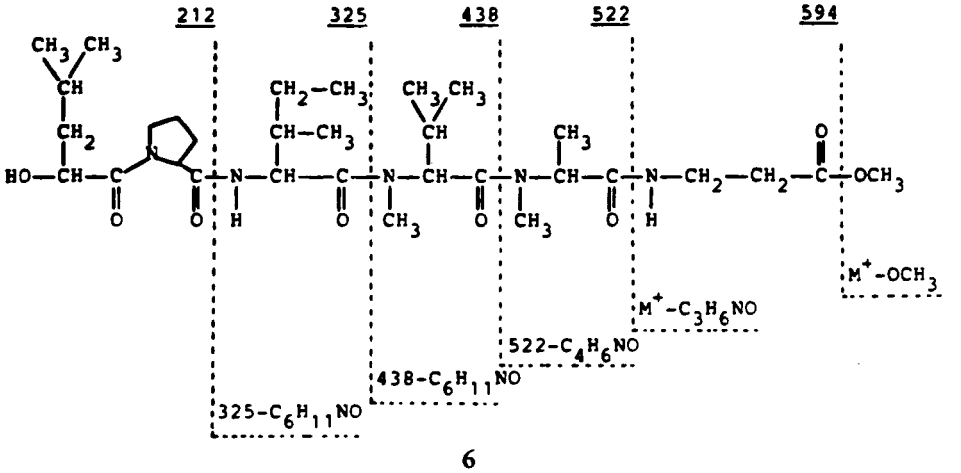

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tive of a destruxin B [3] structure but with one less methylene group. The main difference observed in the ^{1}H nmr of **4**, when compared to the spectra of the two other cyclodepsipeptides, is the presence of an additional N-H absorption (broad doublet, J=6 Hz) at δ 6.24 and the absence of one *N*-methyl signal. Double irradiation experiments show that the N-H proton is coupled to a hydrogen at δ 4.23 (dd, 6, 9.5 Hz) which in turn is coupled to a methine proton at δ 2.05 (m). Irradiation of the multiplet at δ 2.05 causes the collapse of two methyl doublets (δ 1.03, 0.89; d, 7 Hz each) into singlets, showing that the methine proton is part of an isopropyl group. These decoupling experiments show that the N-H proton at δ 6.24 is part of a valinyl group.

Preparation of the hydroxymethyl ester derivative **8** of desmethyldestruxin B and comparison of its hrms with that of the destruxin B ester **6** confirmed the presence of a valine unit in **8**. The loss of the *N*-methylvaline fragment in the hrms of **6** is replaced by the loss of a fragment corresponding to a valinyl group (424-C_5H_9NO) in the spectrum of **8**. These data and comparison with that reported for desmethyldestruxin B [7] (14,17) confirm the identity. The biological activity of homodestruxin B, a new cyclodepsipeptide, and desmethyldestruxin B has not been investigated as yet due to the small amounts of material available to us. It has recently been shown that destruxin B is a host specific toxin.[3]

EXPERIMENTAL

GENERAL PROCEDURE.—H_2O for fungal culturing was distilled in an all glass apparatus prior to use. Solvents for extraction and purification were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62°-70°. E. Merck Si gel 60 (70-230 mesh) was used for column chromatography. For flash and dry flash column chromatography, E. Merck Si gel 60 (230-400 mesh) was employed.

Analytical tlc was carried out on glass plates (75×25 mm or 75×50 mm) coated (approximately 0.3 mm) with Si gel G (Terochem, Edmonton) containing 1% electronic phosphor (General Electric, Cleveland) or E. Merck precoated tlc plates of Si gel 60 F-254 (0.25 mm thickness) or E. Merck DC-Alufolien, kieselgel 60 F-254 (0.2 mm thickness). The chromatograms were visualized under uv light (254 or 350 nm) or by placing them in a chamber containing iodine vapor. Visualization was completed by spraying with 30% H_2SO_4 or with a solution of 5% phosphomolybdic acid containing a trace of ceric sulfate in 5% H_2SO_4, followed by careful charring with a heat gun.

Preparative tlc was carried out on glass plates (20×20 cm, 10×20 cm, 5×7.5 cm) coated (0.35 mm thickness) with Si gel G(Terochem, Edmonton) containing 1% electronic phosphor (General Electric, Cleveland) or E. Merck precoated tlc plates of Si gel 60 F-254 (20×20 cm, 0.25 mm thickness). Materials were detected by viewing under a uv lamp (254 or 350 nm).

Melting points were determined on a Leitz-Weztlar microscope heating stage and are uncorrected. Optical rotations were determined on a Perkin Elmer model 141 polarimeter. Uv spectra were obtained on a Hewlett Packard model 8450A diode array spectrophotometer coupled to a Hewlett Packard 7470A plotter. Ir spectra were recorded on a Nicolet 7199 FT interferometer. Hrms were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS-50 data system. Cims and low resolution eims were obtained using an A.E.I. MS-12 mass spectrometer. Fabms were recorded using an MS-9 spectrometer coupled to a DS-55 data system with NH_3 as ionizing gas. Data are reported as *m/z* (relative intensity). Unless diagnostically significant, peaks with intensities less than 20% of the base peak are omitted.

^{1}H-nmr spectra were measured on a Varian HA-100 spectrometer with a Digilab FTS/NMR-3 data system. High field ^{1}H-nmr and ^{13}C-nmr spectra were recorded on Bruker WH-200 or WH-400 spectrometers with an Aspect 2000 computer system. All nmr measurements employed TMS as an internal standard.

Centrifugation of the concentrated broth was carried out using a Dupont Instruments Sorvall RC-5B refrigerated superspeed centrifuge equipped with a GSA rotor. Amino acid analyses were performed by M. Nattriss, Dept. of Biochemistry, Univ. of Alberta, in a Dionex D500 amino acid analyzer equipped with a Dionex 6A resin, using ninhydrin as the color developing reagent.

Because of the small quantities of material available, molecular formulas were established by mass spectrometry (hrms and cims). Purity of the compounds was established by tlc (in at least two solvent systems) and by the lack of spurious peaks in the ^{1}H- and ^{13}C-nmr spectra.

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[3]J.P. Tewari and P. Bains, Department of Plant Science, University of Alberta, Edmonton, private communication, June 1986.

CULTURING OF *A. BRASSICAE*.—The strain of *A. brassicae* used in these studies[4] was isolated by Dr. J.P. Tewari, Department of Plant Science, The University of Alberta, from infected canola plants (*Brassica campestris* cv. Span) collected near Leduc, Alberta in July 1982. Stock cultures of the fungus were maintained in slant tubes on V-8 juice agar (200 ml V-8 juice, 0.05 g rose bengal, 3 g $CaCO_3$, 20 g agar, 800 ml H_2O) at 4°.

A. brassicae was aseptically transferred from slants to petri dishes containing the V-8 juice agar. The cultures were allowed to grow at room temperature for 10-14 days and then maintained at 4°. Inoculum was prepared by blending a plate of *A. brassicae* with 200 ml of sterile, distilled H_2O. The resulting suspension (10 ml) was used to inoculate liquid media (1 liter).

The fungus was harvested after 20 days of growth. The mycelium was separated from the broth by gravity filtration through cheesecloth. The mycelial mat was dried in a fumehood for 2 days, then extracted for 24 h with Me_2CO in a Soxhlet extractor. The Me_2CO was removed at reduced pressure, and the residue was suspended in H_2O. The resulting suspension was extracted by stirring with an equal volume of EtOAc (2x) for 2 h. The EtOAc extract was dried (anhydrous $MgSO_4$), filtered, and concentrated to give crude mycelial extract (0.22 g/liter).

The broth was concentrated under reduced pressure (bath temperature 35°) to approximately 1/20 of its original volume. A precipitate that appeared at this stage was removed by centrifugation (5000 rpm, 45 min). The concentrated broth was then extracted with EtOAc for 24 h in a liquid-liquid continuous extractor. The EtOAc extract was dried and concentrated to give crude broth extract (0.05 g/liter).

PRELIMINARY FRACTIONATION OF THE CRUDE EXTRACTS.—The crude broth extract was suspended in a minimum amount of H_2O-MeOH (9:1), then partitioned successively between hexane, EtOAc, and *n*-BuOH (3x, ratio solvent: suspension=2:1). Each extract was dried (anhydrous Na_2SO_4), filtered, and concentrated to afford the low, medium, and high polarity crude extracts, respectively. Bioassay of the three extracts showed that the medium polarity fraction, which was the largest fraction, had the strongest biological activity. The medium polarity crude extract was purified by dry flash column chromatography.

LEAF-SPOT BIOASSAY.—A 5% solution of the fraction to be tested is prepared in an organic solvent ($CHCl_3$-MeOH, 80:20). Approximately 20 μl of the test solution is adsorbed in Si gel (1 cm^2, 0.3 mm thickness) and allowed to dry. The impregnated Si gel is placed over a lightly scratched area of a canola leaf (*Brassica napus* L., var. westar) and wetted with approximately 20 μl of distilled H_2O. The leaf is maintained in a petri dish in a moist atmosphere at room temperature. Chlorosis is usually observed within 48 h.

DRY FLASH COLUMN CHROMATOGRAPHY.—The method was developed to separate crude mixtures or complex fractions that are not completely soluble in the eluting solvent. The procedure is a combination of two techniques, dry column chromatography and flash chromatography. The chromatography column is packed as described by Still (18) omitting the sand at the top of the column. The sample, adsorbed on Si gel (ratio sample-adsorbent 1:7) is loaded in a thin, even band, and then covered with approximately 2.5 in of sea sand. A nonpolar solvent is passed through until all of the air is expelled from the column, and the solvent flow rate is adjusted. Elution is then carried out as described for flash chromatography (8) using the appropriate solvent system. We have found that mixtures of Skellysolve B/Me_2CO,Et_2O/Skellysolve B, and C_6H_6/Me_2CO give excellent results for separation of the *A. brassicae* extracts.

ISOLATION OF DESTRUXIN B [**3**].—Separation of the medium polarity broth extract (336 mg) by dry flash column chromatography using gradient elution afforded 17 fractions. The solvent gradient used was Skellysolve B-Me_2CO, 9:1 (1 liter), 17:3 (1 liter), 4:1 (500 ml), 3:1 (500 ml), 7:3 (500 ml), 13:7 (200 ml), 3:2 (200 ml), 11:9 (200 ml), 1:1 (200 ml), 2:3 (200 ml), 3:7 (200 ml), 1:4 (200 ml), 1:9 (400 ml). Fractions (50 ml each) were collected, and like fractions (tlc) were combined. Strong biological activity was observed in fractions 10 (3.5 mg) and 11 (5.6 mg). Both contained the same major component as shown by tlc. Fraction 11 was purified by recrystallization from Skellysolve B/Me_2CO to give destruxin B [**3**] (2.8 mg) as white needles, mp 225-227° [lit. 238° (14)] $[\alpha]_D$ −237° (*c* 0.08, MeOH) [lit. −241° (14)]; ir ($CHCl_3$ cast) 3360, 3290, 1730, 1670, 1630, 1515 cm^{-1}; ^1H nmr ($CDCl_3$) 8.19 (d, 9 Hz, 1 H), 7.17 (d, 9 Hz, 1 H), 5.19 (q, 6.5 Hz; 1 H), 4.94 (d, 11 Hz; 1 H), 4.84 (dd, 3, 10 Hz, 1 H), 4.83 (dd, 9, 6.5 Hz, 1 H), 4.67 (bd, 7 Hz, 1 H), 4.05 (ddd, 13.5, 10, 5, 2 Hz, 1 H), 3.91 (bt, 9 Hz, 1 H), 3.43 (bq, 9 Hz, 1 H), 3.20 (s, 3 H), 3.08 (dddd, 13.5, 11.5, 2, 1.5 Hz, 1 H), 2.73 (s, 3 H), 2.66 (ddd, 18, 11.5, 2 Hz, 1 H), 2.59 (ddd, 18, 5, 1.5 Hz, 1 H), 2.49 (m, 1 H), 2.32 (dsept, 11, 6.5 Hz, 1 H), 2.08 (bs, 1 H), 1.90 (m, 7 H), 1.30 (d, 7 Hz, 3 H), 0.99 (d, 6.5 Hz, 3 H), 0.94 (d, 6.0 Hz, 3 H), 0.93 (d, 6.0 Hz, 3 H), 0.89 (d, 6.5 Hz, 3 H), 0.86 (d, 7.0 Hz, 3 H), 0.86 (t, 7.5 Hz, 3 H); ^{13}C nmr ($CDCl_3$) 173.70 (s), 173.55 (s), 171.08

[4]*A. brassicae* strain UAMH 4936 was deposited with the University of Alberta Microfungus Collection.

(s), 170.93 (s), 169.66 (s, 2×), 71.95 (d), 60.71 (d), 58.13 (d), 55.46 (d), 53.66 (d), 46.44 (t), 38.95 (t), 37.53 (d), 34.42 (t), 33.21 (t), 30.80 (d), 28.85 (t), 28.03 (d), 27.23 (q), 24.49 (t), 24.41 (q), 24.10 (t), 24.32 (q), 21.49 (q), 20.00 (q), 19.62 (q), 15.37 (q), 15.21 (q), 11.32 (q); hrms *m/z* calcd. for $C_{30}H_{51}N_5O_7$ (M^+): 593.3789; found: 593.3798 (7), 536 (30), 508 (23), 451 (22), 423 (22), 86 (100), 70 (60); fabms 1188 (0.1), 594 (22).

DESTRUXIN B METHYL ESTER [6].—Destruxin B [3] (7 mg), MeOH (2 ml), and a catalytic amount of K_2CO_3 were allowed to stir overnight at room temperature. The reaction mixture was poured over ice H_2O (20 ml) and extracted with CH_2Cl_2(4×6 ml). The organic layer was separated, dried, and concentrated to yield 6 mg of crude product which showed a single spot on tlc. Dry flash column chromatography (C_6H_6-Me_2CO, 7:3, 10 cm x 0.4 cm diameter) of the crude product afforded destruxin B methyl ester [6] (4 mg); ir ($CHCl_3$ cast) 3420, 3312, 2950, 1739, 1680, 1631, 1537 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.68 (bt, 5 Hz), 7.41 (bd, 9 Hz), 6.99 (bd, 9 Hz), 6.47 (bt, 5 Hz), 5.20 (d, 11 Hz), 3.62 (s), 3.61 (s), 3.19 (s), 3.00 (s), 2.95 (s), 2.71 (s), 1.29 (d, 8 Hz), 1.21 (d, 8 Hz); hrms *m/z* calcd. for $C_{31}H_{55}N_5O_8$ (M^+): 625.4050; found: 625.4034 (0.7), 522 (1), 438 (25), 325 (7), 212 (22), 86 (100), 70 (42).

O-ACETYLDESTRUXIN B METHYL ESTER.—The methanolysis product of destruxin B [6] (4 mg), Ac_2O (1 ml), and pyridine (0.5 ml) were allowed to stir overnight at room temperature. The reaction mixture was poured into ice H_2O (20 ml) and extracted with EtOAc (3×10 ml). Workup of the organic extract in the usual way gave the crude product (5 mg). Purification by filtration through Si gel gave acetylated destruxin B methyl ester (2.9 mg); ir ($CHCl_3$ cast) 3314, 2961, 1739, 1679, 1641, 1531, 1441, 1241 cm^{-1}; ^1H nmr ($CDCl_3$)[5] δ 7.65 (bt, 6 Hz), 7.46 (bd, 9 Hz), 7.35 (bd, 9 Hz), 6.55 (bt, 5 Hz), 5.29 (d, 11 Hz), 5.18 (d, 11 Hz), 3.67 (s), 3.28 (s), 3.10 (s), 2.97 (s), 2.78 (s), 2.14 (s), 2.12 (s), 1.35 (d, 8 Hz), 1.27 (d, 8 Hz); lrms: 667 (s), 636 (s), 564 (s), 480 (52), 367 (44), 254 (51), 86 (100), 70 (73).

ISOLATION OF HOMODESTRUXIN B [4].—Fractions 11 (5.6 mg) and 12 (3.7 mg), obtained by purification of the medium polarity extract, showed two major components on tlc. Purification of the fractions by preparative tlc yielded both components in pure form. The more polar compound was identified as destruxin B [3]. The less polar compound, homodestruxin B [4], was obtained as a white powder (2.5 mg), mp 202°-204°; ir ($CHCl_3$ cast) 3390, 3300, 2962, 1730, 1672, 1632, 1539, 1515, 1442 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.21 (1 H, d, 9.5 Hz), 7.16 (1 H, d, 9 Hz), 5.15 (1 H, q, 6.5 Hz), 4.99 (1 H, d, 11 Hz), 4.87 (1 H, dd, 10.5, 3 Hz), 4.33 (1 H, dd, 7, 9.5 Hz), 4.64 (1 H, bd, 7.5 Hz), 4.02 (1 H, dddd, 13.5, 10, 5, 2 Hz), 3.87 (1 H, bt, 8.5 Hz), 3.39 (1 H, bq, 9 Hz), 3.16 (3 H, s), 3.03 (1 H, dddd, 13.5, 11.5, 2, 1.5 Hz), 2.69 (3 H, s), 2.62 (1 H, ddd, 18, 11.5, 2 Hz), 2.53 (1 H, ddd, 18, 5, 1.5 Hz), 2.45 (1 H, m), 1.25 (3 H, d, 7 Hz), 0.93 (3 H, d, 7 Hz), 0.89 (3 H, d, 7 Hz), 0.88 (3 H, t, 7 Hz), 0.81 (3 H, t, 7 Hz), 0.80 (6 H, d, 7 Hz); hrms *m/z* calcd. for $C_{31}H_{53}N_5O_7$ (M^+): 607.3945; found: 607.3960 (4), 550 (21), 508 (19), 451 (20), 423 (19), 100 (100), 86 (11), 70 (48).

HOMODESTRUXIN B METHYL ESTER [5].—Homodestruxin B [4] (1 mg), MeOH (1 ml), and a catalytic amount of K_2CO_3 were allowed to stir overnight at room temperature. The reaction mixture was poured over ice H_2O (10 ml) and extracted with CH_2Cl_2 (3×5 ml). The organic layer was separated, dried, and concentrated to produce crude methanolysis product [5] (0.9 mg), tlc: Rf 0.52 (CH_2Cl_2-Me_2CO-MeOH, 70:26:4); ir ($CHCl_3$ cast) 3310, 2959, 1738, 1679, 1630, 1535, 1482 cm^{-1}; hrms *m/z* calcd. for $C_{31}H_{53}N_5O_7$ (M^+ CH_3OH): 607.3945; found: 607.3945 (0.2), 452 (12), 325 (3), 212 (16), 100 (100), 86 (10), 70 (42).

ISOLATION OF DESMETHYLDESTRUXIN B [7].—Fraction 13 (27.7 mg) from purification of the medium polarity crude extract contained a mixture of homodestruxin B [4], destruxin B [3], and a third cyclodepsipeptide. Purification of the fraction by preparative tlc (20×20 cm, $CHCl_3$-MeOH, 96:4, 2×) yielded the cyclodepsipeptides in pure form. The third cyclodepsipeptide was obtained in small amounts (2.6 mg), and was identified as desmethyldestruxin B [7] by comparison of spectra data with published data (14,17); ir ($CHCl_3$ cast) 3390, 3290, 2960, 1735, 1647, 1520, 1180 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$): δ 8.26 (1 H, dd, 2, 8.5 Hz), 7.04 (1 H, d, 9 Hz), 6.24 (1 H, d, 6 Hz), 5.06 (1 H, q, 6.5 Hz), 4.89 (1 H, dd, 3, 11 Hz), 4.62 (1 H, bd, 6.5 Hz), 4.51 (1 H, dd, 6.5, 9 Hz), 4.23 (1 H, dd, 6, 9.5 Hz), 4.00 (1 H, dddd, 13, 10, 5, 2 Hz), 3.91 (1 H, bt, 8.5 Hz), 3.44 (1 H, bq, 9 Hz), 3.16 (1 H, dddd, 13, 11.5, 2, 2 Hz), 2.73 (3 H, s), 2.68 (1 H, ddd, 18, 11, 2 Hz), 2.60 (1 H, ddd, 18, 5, 2 Hz), 2.46 (1 H, m), 1.33 (3 H, d, 7 Hz), 1.03 (3 H, d, 7 Hz), 0.99 (3 H, d, 7 Hz), 0.94 (3 H, d, 6.5 Hz), 0.92 (3 H, d, 7 Hz), 0.90 (3 H, t, 7.5 Hz), 0.89 (3 H, d, 7.5 Hz); hrms *m/z* calcd. for $C_{29}H_{49}N_5O_7$ (M^+): 579.3632; found: 579.3621 (33), 522 (58), 494 (5), 451 (6), 423 (9), 70 (79), 58 (100).

[5]Not all signals are listed because the compound is a mixture of two diastereomers.

DESMETHYLDESTRUXIN B METHYL ESTER [**8**].—Desmethyldestruxin B [**7**] (0.6 mg), MeOH (1 ml), and a catalytic amount of K_2CO_3 were allowed to stir overnight at room temperature. The reaction mixture was poured over ice H_2O (10 ml). Extraction with CH_2Cl_2 (3×5 ml) followed by drying and evaporation of the solvent afforded the crude methanolysis product (0.8 mg). Purification of the crude product by dry flash column chromatography (C_6H_6-Me_2CO, 70:30, 10×0.3 cm column) yielded desmethyldestruxin B methyl ester [**8**] (0.6 mg), tlc: Rf 0.45 (CH_2Cl_2-Me_2CO-MeOH, 70:26:4); ir ($CHCl_3$ cast) 3290, 2960, 1740, 1625, 1539 cm^{-1}; hrms *m/z* calcd. for $C_{30}H_{53}N_5O_8$ (M^+): 611.3894; found: 611.3883 (0.4), 580 (2), 508 (3), 424 (33), 325 (30), 212 (28), 86 (16), 70 (100).

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