JOURNAL OF NATURAL PRODUCTS

Volume 64, Number 5 May 2001

Full Papers

Pseudodestruxins A and B: New Cyclic Depsipeptides from the Coprophilous Fungus *Nigrosabulum globosum*

Yongsheng Che,[†] Dale C. Swenson,[†] James B. Gloer,*[,†] Brenda Koster,[‡] and David Malloch[‡]

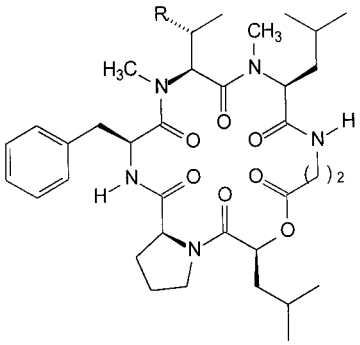
Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1, Canada

Received November 30, 2000

Pseudodestruxins A (**1**) and B (**2**), two new cyclic peptides, have been isolated from cultures of the coprophilous fungus *Nigrosabulum globosum*. The structure of pseudodestruxin A (**1**) was elucidated using 2D NMR techniques and confirmed by single-crystal X-ray diffraction analysis. The structure of **2** was assigned by comparing its NMR and FABMS data with those of compound **1**. The known compounds ascochlorin and 5-chlorocollectorin B were also isolated from *N. globosum*. Although **1** and **2** display antibacterial effects, ascochlorin was found to be responsible for the antifungal activity of the crude extract.

Our studies of coprophilous (dung-colonizing) fungi as sources of antifungal natural products have afforded a variety of new bioactive metabolites.[1] During the course of these continuing investigations, two new cyclic depsipeptides (**1** and **2**) have been isolated from cultures of *Nigrosabulum globosum* Malloch & Cain (Pseudeurotiaceae). We named these compounds pseudodestruxins A and B, respectively, because of structural similarities to the destruxins,[2] a well-known class of fungal metabolites. Two known compounds, ascochlorin[3] and 5-chlorocolletorin B,[4] were also isolated from these cultures. Details of the isolation, structure elucidation, and biological activity of these compounds are reported here.

A subculture of an isolate of *N. globosum* originally obtained from sheep dung was grown in liquid culture. The crude EtOAc extract of the culture broth exhibited antibacterial activity against *Bacillus subtilis* and antifungal activity against *Candida albicans*, *Sordaria fimicola*, and *Ascobolus furfuraceus*. The extract was subjected to silica gel column chromatography, followed by semipreparative reversed-phase HPLC, to afford compounds **1** and **2**, ascochlorin, and 5-chlorocolletorin B.



1 R = CH_2CH_3

2 R = CH_3

Results and Discussion

The molecular formula of pseudodestruxin A (**1**) was determined to be $C_{37}H_{57}N_5O_7$ by HRFABMS analysis [*m/z*, 684.4321 $(M + H)^+$; Δ +1.5 mmu], and the ^1H and ^{13}C NMR data (Table 1) were indicative of a peptide. The ^{13}C and DEPT NMR spectra revealed the presence of nine methylene carbons, eight methyl groups (including two *N*-methyl groups), a phenyl group, and six carboxyl carbonyls (δ

* To whom correspondence should be addressed. Tel: 319-335-1361. Fax: 319-335-1270. E-mail: james-gloer@uiowa.edu.
[†] University of Iowa.
[‡] University of Toronto.

10.1021/np000547r CCC: $20.00
Published on Web 03/30/2001

Table 1. NMR Data for Pseudodestruxins A (**1**) and B (**2**) in $CDCl_3$

	pseudodestruxin A (**1**)			pseudodestruxin B (**2**)
assignment	δ_H (mult., *J* in Hz)	δ_C	HMBC ($^1H \rightarrow \delta_C$)	δ_H (mult., *J* in Hz)
Pro CO		171.4		
α-CH	4.06 (br d, 7.8)	60.8	21.8, 32.2, 171.4	4.07 (br d, 8.4)
β-CH_2	2.21 (m); 2.08 (m)	32.2	21.8, 47.1, 60.8, 171.4	2.22 (m); 2.09 (m)
γ-CH_2	1.72 (m); 1.22 (m)	21.8	60.8	1.73 (m); 1.22 (m)
δ-CH_2	3.46 (m)	47.1	21.8, 32.2, 60.8	3.47 (m)
Phe CO		173.7		
α-CH	4.72 (m)	53.2	35.6, 136.3, 171.4, 173.7	4.73 (m)
β-CH_2	3.01 (dd, 15, 4.8)	35.6	128.8, 136.3	3.01 (dd, 14, 4.8)
	2.89 (dd, 15, 11)		53.2, 128.8, 136.3, 173.7	2.91 (dd, 14, 11)
Ar-1		136.3		
Ar-2/6	7.26 (dd, 7.8, 1.2)	128.8	127.2	7.26 (dd, 7.8, 1.2)
Ar-3/5	7.18 (dd, 7.8, 7.2)	128.7	136.3	7.19 (br t, 7.5)
Ar-4	7.22 (m)	127.2	128.7	7.22 (m)
NH	8.09 (d, 9.6)		171.4	8.10 (d, 8.0)
MeIle/MeVal CO		169.9		
α-CH	5.24 (d, 11)	55.1	24.8, 32.9, 169.9,173.7	5.15 (d, 11)
β-CH	2.22 (m)	32.9	55.1	2.20 (m)
β-Me	0.83 (d, 6.0)	15.7	24.8, 32.9, 55.1	0.86 (d, 6.6); 0.84 (d, 6.6)
γ-CH_2	1.45 (m); 1.06 (m)	24.8	9.5, 15.7, 32.9, 55.1	
γ-Me	0.84 (t, 7.2)	9.5	24.8, 32.9	
N-Me	3.19 (s)	30.2	55.1, 173.7	3.16 (s)
MeLeu CO		168.3		
α-CH	4.96 (dd, 12, 3.0)	58.5	25.3, 28.8, 168.3	4.95 (dd, 13, 2.4)
β-CH_2	2.40 (ddd, 12, 12, 4.0)	39.0	58.5, 168.3	2.38 (m)
	0.98 (m)		168.3	0.99 (m)
γ-CH	1.54 (m)	25.3		1.55 (m)
γ-Me	0.94 (d, 6.6)	23.3	25.3, 39.0	0.95 (d, 6.0)
γ-Me	0.93 (d, 6.6)	23.6	25.3, 39.0	0.94 (d, 6.0)
N-Me	2.84 (s)	28.8	58.5, 169.9	2.85 (s)
β-Ala CO		173.8		
α-CH_2	2.61 (dt, 15, 3.0); 2.55 (m)	35.33	173.8	2.61 (dt, 15, 3.0); 2.55 (m)
β-CH_2	4.12 (m); 3.15 (m)	35.27		4.13 (m); 3.14 (m)
N−H	7.40 (d, 9.6)		168.3	7.45 (d, 10)
leucic acid CO		169.7		
α-CH	5.12 (dd, 11, 1.2)	73.1	24.6, 38.9	5.12 (br d, 11)
β-CH_2	1.93 (m); 1.19 (m)	38.9	24.6, 73.1	1.93 (m); 1.19 (m)
γ-CH	1.88 (m)	24.6	38.9, 73.1	1.89 (m)
γ-Me	0.94 (d, 7.2)	21.7	20.8, 24.6, 38.9	0.94 (d, 6.0)
γ-Me	0.87 (d, 6.6)	20.8	21.7, 24.6, 38.9	0.89 (d, 7.2)

173.8, 173.7, 171.4, 169.9, 169.7, 168.3). An oxymethine signal (*δ* 73.1) accounted for the seventh oxygen atom. The ^1H NMR spectrum displayed signals for two amide *N*-H protons (*δ* 8.09 and 7.40) and two *N*-methyl groups (*δ* 3.19 and 2.84). Proton spin systems were determined by analysis of $^1H-^1H$ COSY and TOCSY spectra recorded at 600 MHz. Shift assignments for carbons bound to hydrogen atoms were established on the basis of HMQC data. Upon extensive analysis of these data, pseudodestruxin A was determined to be a cyclic depsipeptide containing one equivalent each of 2-hydroxyisocaproic acid (leucic acid), phenylalanine (Phe), *β*-alanine (*β*-Ala), proline (Pro), *N*-methylleucine (MeLeu), and *N*-methylisoleucine (MeIle). The amino acid composition of pseudodestruxin A was confirmed by GC−MS analysis of *N*-(trifluoroacetyl) *n*-butyl ester derivatives[5] of the amino acid residues obtained upon acid hydrolysis. HMBC correlations of *N*-methyl groups, amide *N*-H protons, and various α- and *β*-protons with neighboring carboxyl carbons (Table 1) enabled the assignment of each carboxyl carbonyl signal. For example, the α- and *β*-protons of the Pro unit each showed HMBC correlations to a carboxyl carbon signal at *δ* 171.4, leading to assignment of this signal to the Pro carboxyl group. Each *N*-methyl proton signal showed a correlation to the carbonyl of the neighboring acyl group. These and other HMBC data permitted assignment of the amino acid sequence of pseudodestruxin A. The *N*-H proton signal of Phe (*δ* 8.09) showed an HMBC correlation to the carboxyl carbon signal of Pro (*δ* 171.4), indicating that it is acylated by Pro. The *N*-H proton signal of *β*-Ala (*δ* 7.40) was correlated to the carbonyl carbon signal of MeLeu (*δ* 168.3), the MeLeu *N*-methyl signal (*δ* 2.84) was correlated to the carbonyl signal of MeIle (*δ* 169.9), and the MeIle *N*-methyl signal (*δ* 3.19) was correlated with the carbonyl signal of the Phe unit (*δ* 173.7). These correlations enabled assignment of the partial sequence Pro→Phe→MeIle→MeLeu→*β*-Ala. Although no further sequence-relevant HMBC correlations were observed, the only remaining unit (leucic acid) must be placed between Pro and *β*-Ala, thereby completing the structure as shown in **1**. The carboxyl signal for *β*-Ala was located at *δ* 173.8 on the basis of correlations with the corresponding α-methylene signals. The remaining carboxyl signal (*δ* 169.7) showed no HMBC correlations, but could be assigned to the leucic acid unit by default.

Ultimately, the structure of pseudodestruxin A (**1**) was confirmed by single-crystal X-ray diffraction analysis, and a perspective ORTEP plot is shown in Figure 1. The Phe unit was determined to have the L-configuration on the basis of chiral TLC analysis[6,7] of the total acid hydrolyzate. Combining this information with the relative stereochemistry for **1** established by the X-ray data permitted assignment of the absolute configurations for all of the remaining units. All of the amino acid residues in **1** were deduced to have the L-configuration, while the 2-hydroxyisocaproic acid (leucic acid) unit was assigned the *S*-configuration.

HRFABMS data indicated that the minor component (pseudodestruxin B, **2**) contains one CH_2 unit less than **1**. The NMR data for **2** were nearly identical to those of **1**. However, detailed analysis revealed that the ^1H NMR doublet corresponding to the α-H (*δ* 5.24) of MeIle in

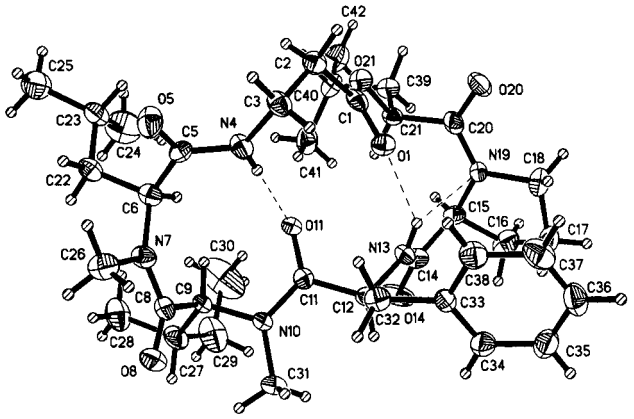


Figure 1. Thermal ellipsoid representation of **1**.

compound **1** was shifted slightly upfield (δ 5.15) in the spectrum of compound **2**. Moreover, the signals corresponding to the γ-CH_2 protons (δ 1.45 and 1.06) of MeIle in compound **1** were absent in the spectrum of **2**, and the γ-CH_3 triplet was replaced by an additional methyl doublet coupled to the β-CH proton resonating at δ 2.20. These observations revealed that the MeIle unit of **1** is replaced by a MeVal unit in **2**. The absolute stereochemistry of **2** was presumed to be analogous to that of **1**.

The structures of the known compounds ascochlorin and 5-chlocollectorin were determined by comparison of their MS and ^{1}H and ^{13}C NMR data with literature values.[3,4]

Pseudodestruxins A (**1**) and B (**2**) exhibited activity in standard disk assays[8] against *B. subtilis* (ATCC 6051), each causing a zone of inhibition of 8 mm at 200 μg/disk. However, both compounds were inactive in assays against the coprophilous fungi *Sordaria fimicola* (NRRL 6459) and *Ascobolus furfuraceus* (NRRL 6460) at the same level. Ascochlorin was found to be responsible for the antifungal activity of the crude extract. It produced a 24 mm zone of inhibition against *C. albicans* (ATCC 90029) and caused 62% and 33% inhibition of radial growth of *S. fimicola* and *A. furfuraceus*, respectively, at 200 μg/disk. It also exhibited activity against *B. subtilis* and *Staphylococcus aureus* (ATCC 29213), affording inhibitory zones of 12 and 11 mm, respectively.

Destruxins are well known for their antiinsectan effects;[9] however, pseudodestruxin A showed no activity in a dietary assay against *Helicoverpa zea* larvae[10] at the 100 ppm dietary level. Although Pro, β-Ala, 2-hydroxyisocaproic acid, Val, and Ile are common components of individual destruxins, no aromatic amino acid units have been reported previously in any members of this class to our knowledge. In addition, all of the known destruxins possess either an Ala or MeAla unit rather than the MeLeu unit found in **1** and **2**.

Only two previous chemical investigations of members of the genus *Nigrosabulum* have been described. These studies resulted in the isolation of a member of the cytochalasin class (from an unidentified *Nigrosabulum* sp.)[11] and caricastatin, a modified dipeptide thiol proteinase inhibitor (from *Nigrosabulum novod*).[12] To our knowledge, chemical studies of *N. globosum* have not been previously reported.

Experimental Section

General Procedures. The melting point was determined using a Fisher-Johns micro melting point apparatus and is uncorrected. The optical rotation was measured on a Jasco model DIP-1000 digital polarimeter, and IR data were recorded using a Mattson Cygnus 25 FT-IR spectrophotometer. GCE-IMS data were recorded at 70 eV using a VG Trio 1 mass spectrometer and a Hewlett-Packard 5890A gas chromatograph equipped with a DB1 capillary column (J & W Scientific, 0.32 mm × 25 m). Chiral TLC analysis was carried out using ChiralPlates (0.25 mm; Macherey-Nagel). High-resolution FABMS data were recorded with a VG ZAB-HF mass spectrometer. ^{1}H and ^{13}C NMR data were obtained with a Bruker AMX-600 spectrometer using the solvent signals ($CDCl_3$; δ 7.24/77.0) as references. Multiplicities of carbon signals were verified through a DEPT experiment. The HMQC and HMBC experiments were conducted using the AMX-600 and were optimized for 150.9 and 8.3 Hz, respectively.

Fungal Material. The isolate of *N. globosum* Malloch & Cain employed in this study was obtained from a sample of sheep dung collected by J. A. Scott in South Australia on March 20, 1994. This isolate was identified by B. Koster and assigned the accession number JS 285 in the D. Malloch culture collection at the University of Toronto. Six 2-L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco) were individually inoculated with two 0.5 cm^2 agar plugs taken from stock cultures of *N. globosum*. Flask cultures were incubated at room temperature on an orbital shaker at 150 rpm for 25 days.

Extraction and Isolation. The culture broth was separated from the mycelium by filtration. The filtered broth (2.4 L) was extracted with EtOAc (4 × 400 mL), and the organic phase was dried over $MgSO_4$ and concentrated to afford 256 mg of brown oil. The crude extract was subjected to Si gel chromatography (50 × 1.5 cm column) using a CH_2Cl_2−MeOH gradient, collecting 20 mL fractions. A fraction of 33 mg that eluted with 100% CH_2Cl_2 was separated by semipreparative reversed-phase HPLC (Alltech HS Hyperprep 100 BDS 8-μm C18; 10 × 250 mm; 90%−100% CH_3CN in H_2O over 20 min) to afford ascochlorin (10 mg). A second fraction (15 mg) eluted with 100% CH_2Cl_2 was separated by reversed-phase HPLC (55%−75% CH_3CN in H_2O over 40 min, then 75%−100% over 15 min) to afford 5-chlorocolletorin B (1.5 mg). A 36 mg fraction eluted with 1% MeOH was further purified by semipreparative reversed-phase HPLC (40−100% CH_3CN over 30 min) on the column described above to afford pseudodestruxin A (**1**; 9.6 mg) and pseudodestruxin B (**2**; 1.2 mg). Crystallization of **1** from acetone produced colorless needles. Ascochlorin and 5-chlorocolletorin B were identified by comparison of their MS, ^{1}H NMR, and ^{13}C NMR data with literature values.[3,4]

Pseudodestruxin A (1): colorless needles; mp 245−251 °C (dec); $[\alpha]_D$ −53° (*c* 0.054, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3325, 3298, 2960, 2931, 2858, 1731, 1688, 1667, 1625, 1571, 1547, 1526 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 684 ([M + H]$^+$; rel int 3), 683 (M$^+$; 12), 626 (7), 583 (99), 565 (19), 554 (22), 526 (38), 493 (32), 468 (10), 370 (10), 337 (5), 259 (15), 174 (23), 126 (53), 100 (100), 84 (14), 70 (73); FABMS (thioglycerol) obsd *m*/*z* 684 ([M + H]$^+$; rel int 100), 557 (8), 529 (3), 486 (4), 430 (1), 426 (3), 382 (3), 370 (5), 273 (2), 224 (12), 194 (22), 168 (13); HRFABMS (thioglycerol/TFA/NH_4Cl) obsd *m*/*z* 684.4321 (M + H)$^+$, calcd for $C_{37}H_{58}N_5O_7$, 684.4336.

X-ray Diffraction Analysis of Pseudodestruxin A (1).[13] Crystals of **1** (0.53 × 0.26 × 0.04 mm) belong to the orthorhombic space group $P2_12_12_1$ with the cell dimensions a = 15.182(5) Å, b = 24.887(8) Å, c = 10.334 (4) Å, and V = 3905(2) Å^3. The 4-NH (β-Ala) atom is involved in an intramolecular H-bond to O11 (Phe), and the 13-NH (Phe) is involved in a bifurcated intramolecular H-bond to O1 (β-Ala) and N19 (Pro). Data were collected on an Enraf-Nonius CAD4 diffractometer (Mo Kα radiation) using θ−2θ scans. The structure was solved using a MULTAN direct methods program and refined using full-matrix least-squares. The 13066 measurements yielded 5067 independent reflections (465 parameters) after equivalent data were averaged and Lorenz and polarization corrections were applied. The final refinement gave R_1 = 0.0441 and wR_2 = 0.0629.

Pseudodestruxin B (2): colorless oil; ^{1}H NMR data, see Table 1; HRFABMS (3-nitrobenzyl alcohol/NaI) obsd *m*/*z* 692.4007 (M + Na)$^+$, calcd for $C_{36}H_{55}N_5O_7Na$, 692.3999.

Acid Hydrolysis of Pseudodestruxin A. A sample of **1** (0.5 mg) was sealed under vacuum in a hydrolysis tube with 1 mL of 6 N HCl and heated at 110 °C for 24 h. The seal was then broken and the solution transferred to a Teflon-lined screw-capped vial and evaporated under a stream of N_2 to afford an oily residue.

Derivatization of the Amino Acid Residues. The acid hydrolyzate was first extracted with ether, and the ether-insoluble residue was dissolved in 1 mL of 3 N HCl in *n*-BuOH and heated at 100 °C for 30 min in a Teflon-lined screw-capped vial. The resulting solution was evaporated under N_2, and the residual solvent was removed by azeotroping with three 1-mL portions of CH_2Cl_2. The oil obtained was dissolved in 0.6 mL of TFA, and 0.3 mL of TFAA was added. The vial was capped tightly and heated at 130 °C for 5 min, then cooled to room temperature. The solvent was removed under dry N_2, and the resulting residue was dissolved in 0.5 mL of CH_2Cl_2 for GC−MS analysis. The derivatives of all the amino acids were identified by interpretation of their GC−EI mass spectra[5] and by comparison with standards. Retention times observed for the derivatives were β-Ala, 3.40 min; MeLeu, 4.47 min; Pro, 4.53 min; Phe, 5.93 min; MeIle, 7.20 min (temperature program: initial $T = 50$ °C for 2 min, then increased to 250 °C at a rate of 20 °C/min).

Chiral TLC Analysis. Prior to derivatization of the amino acid residues, a small amount of the hydrolyzate of **1** and standards of D,L- and L-Phe were each dissolved in a mixture of $CH_3CN-H_2O-CH_3OH$ (4:1:1) and spotted on a chiral TLC plate (Macherey-Nagel). The same solvent system was used as the developing solvent. The Phe in the hydrolyzate cochromatographed with the L-standard (R_f 0.60) and was well resolved from the D-isomer (R_f 0.52).

Acknowledgment. We thank the National Institutes of Health (R01 AI-27436) for financial support of this work. We also thank Dr. Patrick F. Dowd of the USDA NCAUR in Peoria, IL, for conducting the insect assay.

Supporting Information Available: ^1H and ^{13}C NMR spectra and tables of X-ray data for pseudodestruxin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gloer, J. B. In *The Mycota*; IV: Environmental and Microbial Relationships; Wicklow, D. T., Söderström, B. E., Eds.; Springer: New York, 1997; pp 249−268.
(2) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: New York, 1983; pp 631, and references therein.
(3) Kawagishi, H.; Sato, H.; Sakamura, S.; Kobayashi, K.; Ui, T. *Agric. Biol. Chem*. **1984**, *48*, 1903−1904.
(4) Singh, S. B.; Ball, R. G.; Bills, G. F.; Cascales, C.; Gibbs, J. B.; Goetz, M. A.; Hoogsteen, K.; Jenkins, R. G.; Liesch, J. M.; Lingham, R. B.; Silverman, K. C.; Zink, D. L. *J. Org. Chem*. **1996**, *61*, 7727−7737.
(5) Leimer, K. R.; Rice, R. H.; Gehrke, C. W. *J. Chromatogr*. **1977**, *141*, 121−144.
(6) Muramoto, K.; Kamiya, H. *Anal. Biochem*. **1990**, *189*, 223−230.
(7) Liu, T.-Y.; Boykins, R. A. *Anal. Biochem*. **1989**, *182*, 383−387.
(8) Johnson, L. F.; Curl, E. A.; Bond, J. H.; Fribourg, H. A. *Methods for Studying Soil-Microflora-Plant Disease Relationships*; Burgess Publishing: Minneapolis, 1960; pp 71−73.
(9) Cavelier, F.; Verducci, J.; Andre, F.; Haraux, F.; Sigalat, C.; Traris, M.; Vey, A. *Pestic. Sci*. **1998**, *52*, 81−89.
(10) Dowd, P. F. *Entomol. Exp. Appl*. **1988**, *47*, 69−71.
(11) Cameron, A. F.; Freer, A. A.; Hesp, B.; Strawson, C. J. *J. Chem. Soc., Perkin Trans. 2* **1974**, 1741−1744.
(12) Murao, S.; Shin, T.; Katsu, Y.; Iwahara, M.; Hirayama, K. *Agric. Biol. Chem*. **1987**, *51*, 2029−2031.
(13) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

NP000547R