mmol) in DMF (3 mL) was added sodium azide (98.7 mg, 1.52 mmol). The mixture was heated at 80 °C for approximately 48 h under N_2 atmosphere. The mixture was then diluted with ethyl acetate (40 mL), washed with H_2O (3 × 5 mL) and brine solution (2 × 5 mL), dried over Na_2SO_4, and concentrated in vacuo. The product was purified by SiO_2 column chromatography (eluted with 1:1 hexane/ethyl acetate, R_f = 0.21) to afford 72 mg (95%) of the imino ester **4**, as a viscous, yellow oil: $[\alpha]^{25}_D$ = −34.25° (*c* 2.73, $CHCl_3$); IR ($CHCl_3$) 1635 (s), 1720 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.20 (t, 3 H, *J* = 7.1 Hz), 1.30 (s, 3 H), 1.31 (s, 3 H), 1.91–2.02 (m, 2 H), 2.31–2.52 (m, 4 H), 3.81 (br d, 1 H, A of AB q), 3.94 (d, 1 H, B or AB q, *J* = 16.9 Hz), 4.07 (q, 2 H, *J* = 7.1 Hz), 4.67 (m, 1 H), 4.86 (d, 1 H, J = 5.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.13, 20.88, 25.60, 26.80, 29.91, 33.66, 60.20, 64.63, 77.64, 86.52, 111.77, 173.13, 175.84; HRMS ($M^+ - C_2H_5OH$) 209.1052 calcd for $C_{13}H_{21}NO_4(-C_2H_5OH)$, found 209.1049.

(−)-(1*S*,5*R*)-3,3-Dimethyl-8-(3-carboxy-1-propyl)-7-aza-2,4-dioxabicyclo[3.3.0]oct-7-ene (11). The imino ester **4** (884 mg, 3.46 mmol) was dissolved in 33 mL of methanol and 10 mL of water. Potassium carbonate (1.20 g, 8.75 mmol) was added. The mixture was stirred at room temperature for 12 h and was concentrated in vacuo to remove MeOH. The aqueous mixture was washed with ether (1 × 10 mL) and then cooled to 0 °C. After the pH of the solution was adjusted to 3 with 1 N HCl, the solution was saturated with solid sodium chloride. The aqueous layer was repeatedly extracted with ethyl acetate and methylene chloride. The organic extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to give 579 mg (74% yield) of acid **11**, as a yellow solid: mp 105–110 °C $[\alpha]^{25}_D$ = −28.46° (*c* 0.98, $CHCl_3$); IR ($CHCl_3$) 3510 (br), 1720 (s), 1640 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 6 H), 1.91–2.04 (m, 2 H), 2.33–2.38 (m, 2 H), 2.45–2.64 (m, 2 H), 3.88 (br d, 1 H, A of AB q), 3.98 (d, 1 H, B of AB q, *J* = 16.8 Hz), 4.72 (m, 1 H), 4.95 (d, 1 H, *J* = 5.7 Hz), 9.47–9.82 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.81, 25.53, 26.83, 29.74, 33.54, 63.53, 77.24, 86.32, 112.09, 176.31, 177.76.

(−)-(7*S*,8*R*)-7,8-*O*-Isopropylidenedioxy-2-oxo-1-azabicyclo[4.3.0]-non-5-ene (3). A solution of acid **11** (355 mg, 1.56 mmol) in toluene (35 mL) was refluxed with a Dean–Stark trap for 30 h. The solution was then cooled to room temperature, and the solvent was removed in vacuo. The residue was purified by SiO_2 column chromatography (eluted with 1:1 hexane/ethyl acetate, R_f = 0.5 in 10:1 CH_2Cl_2/MeOH) to provide 284 mg (87%) of enamide **3**: $[\alpha]^{25}_D$ = −86.70° (*c* 1.82, $CHCl_3$); IR ($CHCl_3$) 1650 (s), 1680 (sh) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 3 H), 1.45 (s, 3 H), 2.34–2.57 (m, 4 H), 3.70 (dd, 1 H, *J* = 5.5 and 13.1 Hz), 3.86 (d, 1 H, *J* = 13.1 Hz), 4.73 (br t, 1 H), 5.03 (d, 1 H, J = 5.9 Hz), 5.24 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.85, 25.69, 27.44, 30.44, 49.95, 75.88, 79.30, 101.59, 112.76, 139.95, 168.50; HRMS (M^+) 209.1052 calcd for $C_{11}H_{15}NO_3$, found 209.1047.

(−)-(1*S*,2*R*,8*R*,8a*R*)-1,2-*O*-Isopropylidenedioxy-8-hydroxyindolizidine (Swainsonine Acetonide; 2). To a cold (0 °C) solution of enamide **3** (261 mg, 1.29 mmol) in anhydrous THF (1.6 mL) was added 5.0 mL of 1.0 M BH_3·THF solution. The reaction was brought to room temperature overnight. The solvent was removed in vacuo, and ethanol (3 mL) was then added. To this solution were added sodium hydroxide (208 mg, 5.20 mmol) and 30% hydrogen peroxide (0.6 mL). An additional 2 mL of ethanol was added and the mixture refluxed for 2 h. The mixture was cooled, the ethanol removed in vacuo, and the residue dissolved in 3 mL of H_2O. The aqueous solution was saturated with solid NaCl and then extracted five times each with ethyl acetate and methylene chloride. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give crude **2** as a colorless solid. Purification by SiO_2 column chromatography (eluted with 25:1 CH_2Cl_2/MeOH) afforded 209.1 mg (79%) of the pure product (visualized with I_2) as a white crystal: mp 100–103 °C; $[\alpha]^{25}_D$ = −72.76° (*c* 0.43, MeOH) [lit.[3a] $[\alpha]^{24}_D$ = −75.1° (*c* 1.54, MeOH)]; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.18–1.24 (m, 1 H), 1.33 (s, 3 H), 1.51 (s, 3 H), 1.61–1.70 (m, 4 H), 1.85 (m, 1 H), 2.05 (m, 1 H), 2.13 (dd, *J* = 4.2 and 10.7 Hz, 1 H), 2.98 (dt, *J* = 3.2 and 10.6 Hz, 1 H), 3.16 (d, *J* = 10.7 Hz, 1 H), 3.81–3.87 (m, 1 H), 4.61 (dd, *J* = 4.2 and 6.2 Hz, 1 H), 4.71 (dd, *J* = 4.6 and 6.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.08, 24.81, 25.96, 33.01, 51.60, 59.88, 67.53, 73.68, 78.26, 79.17, 111.37; HRMS (M^+) 213.1365 calcd for $C_{11}H_{19}NO_3$, found 213.1366.

(−)-(1*S*,2*R*,8*R*,8a*R*)-1,2,8-Trihydroxyoctahydroindolizine (Swainsonine; 1). To a solution of acetonide **2** (104 mg, 0.49 mmol) in THF (6.5 mL) was added 6.0 mL of 6 N HCl. The colorless solution was stirred overnight at room temperature. The solvent was removed in vacuo, leaving a colorless, viscous oil. The oil was then purified by ion-exchange chromatography (Dowex-1X8). Fractions (visualized with iodine or ninhydrin) were collected and concentrated in vacuo to furnish 72 mg (85% yield) of **1** as a white solid: mp and mixed mp 140–142 °C [lit.[3a] mp 144–145 °C]; $[\alpha]^{25}_D$ = −75.71° (*c* 2.33, MeOH) [lit.[3a] $[\alpha]^{25}_D$ = −87.2° (*c* 2.1, MeOH); lit.[4b] $[\alpha]^{25}_D$ = −78.9° (*c* 1.14, MeOH)]; R_f = 0.36 in 1-butanol/chloroform/methanol/concentrated ammonium hydroxide (4:4:4:1); ^1H NMR (D_2O, ref DSS, 300 MHz) δ 4.34 (m, 1 H, H-2), 4.24 (dd, $J_{1,8a}$ = 3.7 Hz, $J_{1,2}$ = 6.1 Hz, 1 H, H-1), 3.78 (ddd, *J* = 3.9, 9.3, and 10.7 Hz, 1 H, H-8), 2.89 (m, 1 H), 2.86 (dd, *J* = 2.6 and 11.0 Hz, 1 H, H-3), 2.53 (dd, *J* = 7.8 and 11.0 Hz, 1 H, H-3′), 2.04 (m, 1 H), 1.96 (m, 1 H), 1.89 (dd, $J_{8a,8}$ = 9.3 Hz, $J_{8a,1}$ = 3.7 Hz, H-8a), 1.70 (m, 1 H), 1.49 (m, 1 H), 1.22 (m, 1 H); ^{13}C NMR (D_2O, ref CH_3CN, 100 MHz) δ 23.21, 32.51, 51.72, 60.65, 66.37, 69.08, 69.72, 72.87; HRMS (M^+) 173.1052 calcd for $C_8H_{15}NO_3$, found 173.1041.

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Registry No. 1, 72741-87-8; **2**, 85624-09-5; **3**, 119011-32-4; **4**, 119011-33-5; **6**, 51607-16-0; **7**, 119011-34-6; **8**, 119011-35-7; **11**, 119011-36-8; **13**, 119011-37-9; $Br^-Ph_3P^+(CH_2)_3CO_2Et$, 50479-11-3.

Theonellamide F. A Novel Antifungal Bicyclic Peptide from a Marine Sponge *Theonella* Sp.[1]

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Abstract: A novel antifungal peptide, theonellamide F, was isolated from a marine sponge, genus *Theonella*. It is a dodecapeptide composed of L-Asn, L-*a*Thr, two residues of L-Ser, L-Phe, β-Ala, (2*S*,3*R*)-3-hydroxyasparagine, (2*S*,4*R*)-2-amino-4-hydroxyadipic acid, τ-L-histidino-D-alanine, L-*p*-bromophenylalanine, and (3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-(*p*-bromophenyl)-5,7-octadienoic acid. Its bicyclic structure including absolute stereochemistry was unequivocally determined as **1**, which contains an unprecedented histidinoalanine bridge.

Marine sponges of the family Theonellidae, which includes the genera *Theonella* and *Discodermia*, have proved to be a source of metabolites with interesting biological activities as well as chemical structures. Compounds derived from this family are roughly divided into two classes:[2] (a) peptides, discodermins from

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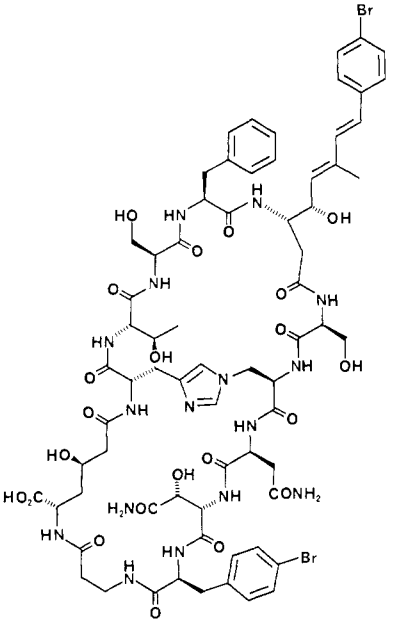
Discodermia kiiensis,[3] and theonellapeptolides from *Theonella* sp.[4,5] and (b) aliphatic compounds, swinholide A from *Theonella swinhoei*,[6] bistheonellides from *Theonella* sp.,[7,8] and calyculins from *Discodermia calyx*.[9] In the course of our study on bistheonellides,[7] we found that the same sponge contained water-soluble antifungal compounds. Isolation and structural study of the major antifungal metabolite, theonellamide F, are described in the present report.

Results and Discussion

The frozen sponge was extracted with EtOH, MeOH, and 50% aqueous *n*-PrOH. The combined extracts were evaporated, and the residual aqueous suspension was extracted with ether. The aqueous phase was purified by TSK G3000S, silica gel column chromatography, and reverse-phase HPLC to obtain theonellamide F (**1**; 9×10^{-3}% based on wet sponge).[10] Theonellamide F



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inhibited growth of various pathogenic fungi (*Candida* spp., *Trichophyton* spp., and *Aspergillus* spp.) at concentrations of 3–12 μg/mL. It was also cytotoxic against L1210 and P388 leukemia cells with IC_{50} of 3.2 and 2.7 μg/mL, respectively.

Theonellamide F showed UV maxima at 283 nm (ε 24 400), 294 (24 800), and 315 (sh) (13 600). A FAB mass spectrum gave a complex multiplet between *m/z* 1649 and 1655 with an intensity ratio of 1:2:1.6 for peaks at *m/z* 1649, 1651, and 1653, suggestive of two bormine atoms. A peptide was inferred from the generation of several ninhydrin-positive spots on TLC upon acid hydrolysis (6 N HCl, 110 °C, 16 h). Amino acid analysis of the hydrolysate revealed the presence of one residue each of Asp, *a*Thr, Phe, and βAla, and two residues of Ser and ammonia. There were several unknown peaks in the chromatogram: an acidic amino acid (designated as X-1), diastereomeric acidic amino acids in 1:1 ratio (X-2a and X-2b), diastereomeric neutral amino acids in 1:2 ratio (X-2b-lactone and X-2a-lactone),[11] a basic amino acid with a strong ninhydrin coloration (X-3), and a broad peak with retention time comparable to Trp (X-4). Additionally, the ether extract of the hydrolysate contained an inseparable complex mixture of UV-absorbing compounds, perhaps decomposition products of the chromophore.

The stereochemistry of the standard amino acid residues were determined by chiral GC–MS analysis, which allowed us to assign the L configuration for the Asp, Ser, *a*Thr, and Phe residues. The GC–MS analysis implied the presence of a 1:1 diastereomeric pair of aminohydroxyadipic acids and bromophenylalanine.

In order to study the structures of X-1–X-4, the acid hydrolysate was subjected to ion-exchange chromatography, which achieved successful separation of all constituents.

X-1 gave a greenish-gray color with ninhydrin, a feature characteristic of an aminohydroxydicarboxylic acid.[12] The ^1H NMR and the ^{13}C NMR spectra revealed two contiguous methines substituted by nitrogen and oxygen and two carboxyl carbons. These data indicated that X-1 was β-hydroxyaspartic acid, which was supported by the MH^+ ion at *m/z* 150 in the FAB mass spectrum. (2*S*,3*R*) stereochemistry was deduced from comparing optical rotation values with those of the four stereoisomers reported in the literature.[13] Thus, X-1 was (2*S*,3*R*)-3-hydroxyaspartic acid.

Surprisingly, X-2a and X-2b, which appeared between the Asp and Glu peaks in the amino acid analysis, eluted after βAla as a single peak in preparative ion-exchange chromatography. The FAB mass spectrum showed the MH^+ ion at *m/z* 178, suggesting that X-2 was a diastereomeric mixture of aminohydroxyadipic acids, whose presence had been inferred from GC-MS analysis. The ^1H and ^{13}C NMR spectra revealed that the isolated material was a 1:2 mixture of triethylammonium salts of 2-amino-4-hydroxyadipic acids, which were spontaneously converted to an equilibrium mixture of γ-lactones and dicarboxylic acids in acidic or neutral media.[14] Most of the X-2a and X-2b formed γ-lactones under our chromatographic conditions. It is also likely that triethylamine opened up the lactones to form the triethylammonium salts of dicarboxylic acids during evaporation of triethylammonium acetate buffer. Treatment of the isolated mixture of X-2a and X-2b with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile(BocON)/Et_3N followed by methylation with CH_2N_2 and HPLC separation on silica gel afforded methylated Boc-X-2a-lactone (**2**) and Boc-X-2b-lactone (**3**). The major isomer **2** appeared to be the *cis*-lactone judging from the ^1H NMR data of the synthetic model compounds **4** and **5**.[17] The stereochemistry of C-2 was assigned to be L (2*S*) on account of

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(10) The sixth peak in the reverse-phase HPLC analysis (YMC AM-ODS, 32% *n*-PrOH/20 mM KH_2PO_4) of the antifungal fraction was termed theonellamide F.

(11) The integration of X2-lactone pair in the amino acid analysis was less than half of that of X2 doublet peaks; the sum total of the four peaks (X2a, X2b, and X2a- and X2b-lactones) roughly corresponded to one amino acid residue.

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(13) $[\alpha]_D$ value of +53.0° was reported for the (2*S*,3*R*) isomer, and +1.3° for the (2*S*,3*S*) isomer: Kaneko, T.; Katsura, H. *Bull. Chem. Soc. Jpn.* **1963**, *36*, 899–903.

(14) Although X2a and X2b lactonized slowly in aqueous solution, NMR data were assignable to a mixture of dicarboxylic acids because of the high-field ^1H NMR shift of H-4 (δ 3.98) as well as the chemical shifts equivalence of H-2 methylene protons. For 2-amino-4-hydroxyadipic acid, the synthesis of a mixture of four stereoisomers was described,[15] but its natural occurrence has not been reported. A report on its possible occurrence in the culture medium of *Vibrio cholerae* was based virtually only on elemental analysis.[16]

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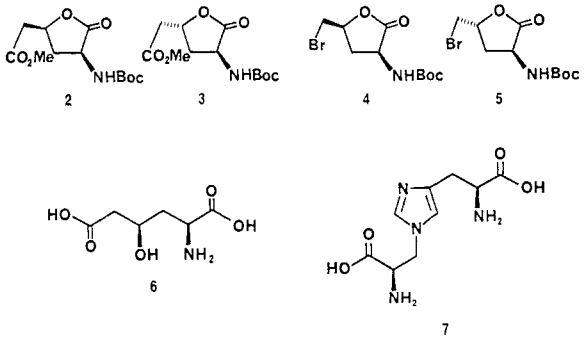


2 3 4 5

6

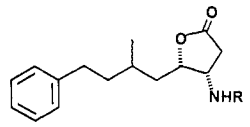
7

a positive Cotton effect at 217 nm.[18] Accordingly, the *cis*-lactone had (2*S*,4*S*) stereochemistry, while the minor *trans*-lactone **3**, as deduced from the ^{1}H NMR spectrum, must have the (2*S*,4*R*) stereochemistry. The C-4 hydroxyl group might have been epimerized through a backside attack of the carboxyl group during lactonization. The ratios of 4*S* to 4*R* isomers were 1:4, 1:2, and 1:1.5, when the acid hydrolysis was carried out for 2, 4, and 8 h, respectively, as analyzed by chiral GC–MS analysis. Therefore, we concluded that (2*S*,4*R*)-2-amino-4-hydroxyadipic acid (**6**) was the constituent of theonellamide F, while the (2*S*,4*S*) isomer was an artifact.

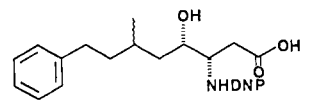
X-3 had a molecular weight of 242 as evidenced by the MH^+ ions in the FAB and FD mass spectra. Although it looked like a single compound from the amino acid analysis and the ^{13}C NMR spectrum, the ^{1}H NMR spectrum and degradative work clearly demonstrated that X-3 was a 1:1.2 diastereomeric mixture.[19] Identification of histidyl and β-substituted alanyl units was straightforward from the spectral data. The ^{1}H and ^{13}C NMR shifts in the β-position of the substituted Ala unit [δ_H 4.56 (2 H, d, J = 5.1 Hz), δ_C 47.6 (t)] indicated that the carbon was attached to one of the nitrogens of the imidazole ring.[20] Degradation of X-3 with $KMnO_4/NaIO_4$ (room temperature, 5 min) followed by derivatization and chiral GC–MS analysis led to L-Asp and approximately a 1:1 mixture of L- and D-2,3-diaminopropionic acid (Dpr). This result established that the β-substituted alanyl unit, whose α-carbon was partially epimerized, was linked to one of the nitrogens of the imidazole ring. The position of substitution was corroborated by ^{1}H–^{1}H difference NOE experiments: irradiation of the imidazole H-5 at 7.09 ppm induced NOEs for the α-CH and β-CH_2 of both His and Ala units, while irradiation of the imidazole H-2 only enhanced the α-CH and β-CH_2 of the substituted Ala unit. Thus, the β-carbon of the substituted Ala must be linked to the τ-nitrogen of the imidazole ring. The stereochemistry of the Ala unit in the intact peptide was elucidated as follows. Theonellamide F was hydrolyzed with 6 N HCl at 107 °C for 8 h, followed by oxidation with $KMnO_4/NaIO_4$ to afford a 1:3 mixture of L- and D-Dpr. Consequently, X-3 was present in the intact peptide as τ-L-histidino-D-alanine (**7**).

X-4, the last unidentified amino acid of the hydrolysate, must be an isomer of bromophenylalanine, as suggested by the GC–MS analysis. Since the presence of a para-disubstituted benzene ring was indicated by the ^{1}H NMR spectrum of X-4, comparison of X-4 with authentic DL material by TLC and amino acid analysis was performed, which showed their identity. Theonellamide F was hydrogenated (H_2/Pd–C) and hydrolyzed to yield two residues of L-Phe; X-4 was no longer present. Hence, X-4 was L-*p*-bromophenylalanine.

Since the amino acid residues discussed so far accounted for a molecular weight of 1327/1329, the remaining portion must have a molecular weight of 321/323, including one bromine atom. This portion, though quite labile under acidic conditions, could be stabilized by hydrogenation. A 5:1 mixture of compounds with an M^+ at m/z 471 (GC–MS) resulted from acid hydrolysis of hydrogenated **1**, followed by derivatization. In addition, the TFA trideuteriomethyl ester derivatives and the TFA *n*-butyl ester derivatives gave M^+ peaks at m/z 474 and 513, respectively. Therefore, the products were monocarboxylic acids. The presence of fragment ions in the EIMS corresponding to [M^+ – CF_3CONH], [$M^+ - CF_3CO_2$], and [$M^+ - CF_3CO_2 - CF_3CONH$] suggested a molecular weight of 265 for the underivatized amino acids. A prominent base peak at m/z 91 was indicative of the presence of a benzene ring. Furthermore, a UV-absorbing lactone [HX-5-lactone (**8**); H stands for hydrogenated] was separated



8 R=H
9 R=DNP



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by preparative TLC from the EtOAc-soluble portion of the acid hydrolysate of the hydrogenated (H_2/Pd–C) peptide. This lactone had a molecular weight of 247 (FABMS), indicating a loss of H_2O from the intact hydrolysis product, as predicted from the GC–MS analysis. Although ^{1}H NMR analysis led us to assign the gross structure of HX-5-lactone, broad ^{1}H NMR signals and instability hampered stereochemical studies. This was overcome by preparing a 2,4-dinitrophenyl derivative of **8**. The DNP derivative **9** possessed 3,4-syn stereochemistry, which was verified by the proximity of the N*H* and H_2-5 as well as H-3 and H-4 in ^{1}H–^{1}H NOE experiments. Since the difficulty of conformation analysis for 2-unsubstituted γ-lactones is known,[21] we attempted to deduce the absolute configuration of C-3 by applying CD spectroscopy to the carboxylic acid **10**, which was prepared by hydrolysis of **9** with K_2CO_3. The CD spectrum exhibited a positive maximum at 410 nm and negative maximums at 340 and 285 nm, as reported in the literature;[22] thus, (*S*) stereochemistry was assigned to C-3. Therefore, **8** had (3*S*,4*S*) absolute configuration. HX-5-lactone was derived from the chromophoric amino acid of theonellamide F, which has two (*E*)-olefins conjugated to a *p*-bromobenzene ring, as described below. Accordingly, the structure of the acid-labile moiety in the peptide was (3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-(*p*-bromophenyl)-5,7-octadienoic acid (Aboa), which fully accounted for the missing portion.

In summary, theonellamide F contains one each residue of L-Asp, L-*a*Thr, L-Phe, βAla, (2*S*,3*R*)-3-hydroxyaspartic acid (L-*erythro*-β-hydroxyaspartic acid) (OHAsp), (2*S*,4*R*)-2-amino-4-hydroxyadipic acid (Ahad), τ-L-histidino-D-alanine, L-*p*-bromophenylalanine (BrPhe), and Aboa, and two residues of L-Ser.

Following the complete determination of all component amino acids, the structural study of the intact molecule was initiated. Theonellamide F was negative to ninhydrin reagent, thereby suggesting the absence of an N terminus. The presence of two primary amides was shown by the liberation of 2 equiv of ammonia upon acid hydrolysis and by the presence of two characteristically coupled primary amide protons in the ^{1}H NMR spectrum.[23] There was one free carboxylic acid group, which was implied by

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(19) (a) Partial exchange of the H-2 of the histidyl imidazole unit in proteins with deuterium in a slightly basic deuteriated medium was reported: (Arata, Y.; Shimizu, A.; Matsuo, H. *J. Am. Chem. Soc.* **1978**, *100*, 3230–3232. Freshly prepared D_2O solution of X3 demonstrated the H-2 signal with an intensity comparable to that of the H-5 signal. (b) It is likely that the α-carbon of α,β-dinitrogenous amino acids tends to epimerize under total acid hydrolysis conditions, since most of the α-proton of the Dpr suffered exchange with deuterium during hydrolysis in 6 N DCl in D_2O (110 °C, 16 h), as is case with 2,3-diaminobutyric acid: Bodanszky, A. A.; Bodanszky, M. *J. Antibiot.* **1970**, *23*, 149–154. However, no epimerization of Dpr was observed under acidic derivatization condition for the GC–MS analysis.

(20) For the NMR data of methylene groups attached to the nitrogen of imidazole, see: Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003–3006.

(21) Angelotti, T.; Krisko, M.; O'Connor, T.; Serianni, A. S. *J. Am. Chem. Soc.* **1987**, *109*, 4464–4472.

(22) (a) Nagai, U.; Kawai, M.; Yamada, T.; Kuwata, S.; Watanabe, H. *Tetrahedron Lett.* **1981**, *22*, 653–654. (b) Kawai, M.; Nagai, U.; Katsumi, M.; Tanaka, A. *Tetrahedron* **1978**, *34*, 3435–3444.

(23) Kessler, H.; Steuernagel, S.; Gillessen, D.; Kamiyama, T. *Helv. Chim. Acta* **1987**, *70*, 726–741.

methylation with CH_2N_2, resulting in an increase of 14 mass units in the FAB mass spectrum. Furthermore, the number of free hydroxyl groups was estimated to be five, since theonellamide F yielded a pentaacetate (FABMS) upon treatment with Ac_2O/pyridine. The presence of five hydroxyl groups implied that the peptide had no ester function, which was supported by the absence of an ester band in the IR spectrum. Hence, theonellamide F must be a cyclic peptide. Accurate mass measurements in the FAB mass spectrum established a molecular formula of $C_{69}H_{86}N_{16}O_{22}Br_2$, which supported a cyclic peptide structure with two primary amide and one carboxylic acid groups in the side chains.

Attempts at partial hydrolysis under various acidic conditions and digestion with pronase were unsuccessful, so that the sequence study was conducted by NMR spectroscopy.[24] Theonellamide F gave broad unresolved ^1H NMR signals at room temperature both in D_2O and in DMSO-d_6; however, signals were sharpened at 60 °C in both solvents.

First of all, the signals for the Aboa unit were assigned by interpreting the HOHAHA,[25] NOESY, ROESY,[26] HMQC,[27] and HMBC[27] spectra. There was a para-disubstituted benzene ring, whose protons showed ROESY correlation with (*E*)-olefinic protons [δ 6.74 (d, J = 16.1 Hz), 6.52 (d, J = 16.1 Hz)], which in turn were correlated with an olefinic methyl [δ 1.74 (3 H, s)] and an olefinic proton [δ 5.39 (1 H, d, J = 8.4 Hz)] in the ROESY spectrum. The intense NOESY cross peaks between the signals at δ 6.74 and 5.39 and between δ 6.52 and 1.74 suggested (*E*,*E*) geometry. Interpretation of the HOHAHA spectrum starting from the olefinic proton at δ 5.39 led to a further structural unit CH(OH)CH(NH)CH_2CO [δ 4.28 (1 H, m, C*H*OH), 4.19 (1 H, m, C*H*NH), 7.49 (1 H, m, NH), 2.25 (1 H, m), 2.35 (1 H, m)]. Thus the presence of the Aboa unit in the intact peptide was secured. This was fully supported by ^{13}C NMR signals assigned by HMQC and HMBC spectra.

A study of the spin systems of component amino acids revealed that there were one each of ABX (OHAsx), A_3MNX (αThr), ABMNX (βAla), ABMCDNX (Ahax), and ABM(X)NP (Aboa), seven ABMX [Asx, Ser (2), sHis, sAla, Phe, and BrPhe] systems, an imidazole (sHis), a monosubstituted benzene ring (Phe), two para-disubstituted benzene rings (BrPhe and Aboa), and signals for the remaining olefinic portion of Aboa. All ^1H NMR signals, except for those of seven ABMX systems and two para-disubstituted benzene rings, were unambiguously assigned by the phase-sensitive COSY spectrum in DMSO-d_6 and HOHAHA spectra in DMSO-d_6, D_2O, and 90% H_2O/D_2O. Two para-disubstituted benzene rings were distinguishable in the NOESY spectrum, in which the β-protons of BrPhe and ξ- and η-protons of Aboa displayed NOE cross peaks with the respective aromatic protons. The spin systems of Phe, sHis, and sAla were assigned from their β-protons, which gave cross peaks with the respective aromatic protons of each residue in the NOESY spectrum. Among the remaining three ABMX systems, β-protons of two Ser residues appeared in the lower field, leaving only Asx residue. Because of the poor solubility in D_2O, no C–H correlation was observed in aqueous media, whereas HMQC and HMBC spectra could be obtained in DMSO-d_6 solution. Assigned ^{13}C NMR data fully supported the deduced amino acid composition.

Sequence analysis was performed by interpreting primarily the NH–α-H, NH–β-H_2, and NH–NH region of the NOESY data in 90% H_2O/D_2O, and ROESY and NOESY spectra in DMSO-d_6.[28] Since the amide proton signals were better separated in 90% H_2O/D_2O solution, in which the only overlapping NH signals were those of Ahax and βAla, NOESY data in aqueous medium were interpreted first (Figure 1).

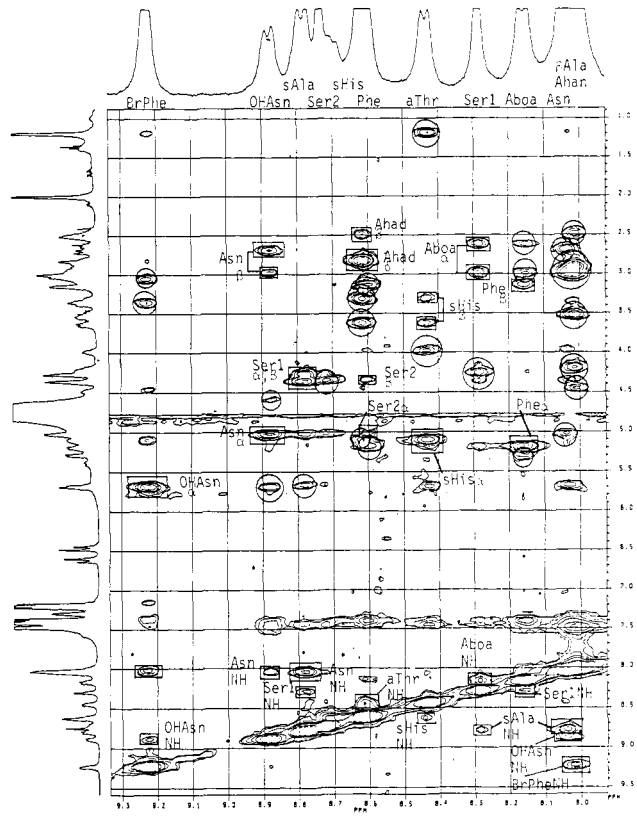

Figure 1. NH signal region of the NOESY spectrum of theonellamide F in 90% H_2O/D_2O. Cross peaks due to intraresidual NOEs were encircled, while those of interresidual NOEs used in sequence analysis were boxed.

A partial sequence Ser(II)-Phe-Aboa-Ser(I)-sAla-Asx-OHAsx-BrPhe was deduced starting from the NH of BrPhe. The NH of BrPhe gave cross peaks with α-H and NH of the OHAsx residue, while the NH of OHAsx was correlated with NH, α-H, and β-H_2 of the Asx residue. The NH of Asx showed a strong cross peak with NH of sAla, which was in turn correlated with NH, α-H, and β-H_2 of the Ser(I) residue. Meanwhile, NH of Ser(I) gave strong cross peaks with α-H_2 and NH of Aboa, whose NH showed interrelation with α-H and β-H_2 of the Phe residue. The Phe residue was further linked to the Ser(II) residue, which was corroborated by the correlation between NH of Phe and α-H of Ser(II). However, NH of Ser(II) gave no sequential cross peaks.

On the other hand, another partial sequence, isoAhax-sHis-αThr, was deduced starting from the NH of the αThr residue, which interacted with NH, α-H, and β-H_2 of the sHis residue. Furthermore, the NH of the sHis residue gave cross peaks with δ-H_2 and β-H_2 of Ahax residue, among which the cross peak with δ-H_2 was more intense, thus indicating the presence of an iso-linked Ahax residue. Further sequence assignment was not possible due to overlap of NH signals of the isoAhax and βAla residues.

Complementary data were obtained from the ROESY spectrum in DMSO-d_6, in which the amide protons of isoAhax and βAla were well separated. First, NH of the Ser(II) residue was correlated with α-H and β-H of the αThr residue, thereby connecting the above two partial sequences. The only unassigned residue, βAla, was inserted between isoAhax and BrPhe residues, since NH of isoAhax showed cross peaks with α-H_2 and one of the β-H_2s of the βAla residue; the NH proton of the βAla residue was in turn correlated with NH, α-H, and β-H_2 of the BrPhe residue. Other signals in the NH regions of ROESY and NOESY spectra in DMSO-d_6 fully supported the sequential assignment made by interpretation of the NOESY spectrum in 90% H_2O/D_2O.

At this point, locations of two primary amides and one carboxylic acid were not firmly established from the NOE data. Moreover, it was not yet known whether Asx and OHAsx were α-linked or iso-linked, though the fact that both α-protons of the

(24) In the NMR study, the histidinoalanine portion was considered as two amino acid residues, sHis and sAla, linked by the side chains.

(25) Edwards, M. W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918–923.
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(27) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
(28) Wuthrich, K. *NMR of Proteins and Nucleic* Acids; Wiley: New York, 1986.

two residues displayed stronger NOESY cross peaks with the NH signals of their adjacent residues than did the β-protons favored α-linkages for both residues. In order to construct a total structure, theonellamide F was treated with [bis(trifluoroacetoxy)iodo]-benzene, which converts primary amides into amines. The reaction product was subjected to total acid hydrolysis, followed by amino acid and GC–MS analyses. These analyses showed the presence of Ahad, which indicated that the α-carboxyl group of the isoAhax residue was free in the peptide. On the other hand, the presence of Dpr in the hydrolysate indicated that the Asn residue was α-linked. The presence of an α-linked OHAsn residue was shown by the HMBC spectrum of the intact peptide in which the primary amide protons resonated at 7.18 ppm; this showed NOESY correlation with β-H of this residue as it gave a cross peak with C-β at 71.1 ppm.

Several puzzling NOESY and ROESY cross peaks were informative for the shape of the molecule. There were two signals that showed several through-space interactions. One was the α-H of OHAsn, which gave NOESY cross peaks with α-H and β-H_2 of sHis and γ-H_3 of *a*Thr. The other was the γ-H_3 of *a*Thr, which interacted with the protons on all three benzene rings, NH and β-H_2 of BrPhe, NH and β-H_2 of Asn, and α-H of OHAsn. Moreover, methyl protons of Aboa gave NOESY cross peaks with aromatic protons of Phe and BrPhe. These results indicated that the bicyclic peptide had a cagelike shape, in which the three benzene rings and the methyl group of *a*Thr were spatially close, whereas OHAsn and sHis were a short distance from the peptide backbone.

Theonellamide F contains five unusual amino acid residues. (2*S*,3*R*)-3-Hydroxyaspartic acid was reported as a constituent of vitamin K dependent proteins[29] and of xylocandins,[30] whereas the other four amino acids are, to the best of our knowledge,[31,32] the first to be isolated from natural sources. Particularly interesting is the presence of Aboa and histidinoalanine. β-Amino-phenyldecanoic acids, biogenetically related to Aboa, have been reported as constituents of cyclic peptides of blue-green algae,[33,34] while the presence of a bridge between the nitrogen of the histidyl imidazole and C-2 of the tryptophenyl indole was reported in moroidin, a toxic peptide of an Australian plant *Laportea moroides*.[35]

Experimental Section

General Information. Infrared spectra were recorded on a Jasco IR-G spectrometer. Ultraviolet spectra were measured on a Hitachi 330 spectrophotometer. Optical rotations were determined with a Jasco DIP-140 polarimeter. CD spectra were recorded on a Jasco J-20C spectropolarimeter. ^1H and ^{13}C NMR spectra of the degradation products were measured on either JEOL FX100, JEOL GX400, or Bruker AM500 NMR spectrometers. Mass spectra, except high-resolution FAB mass spectra, were recorded on a JEOL DX303 mass spectrometer. High-resolution FAB mass spectra were measured on a JEOL HX110 mass spectrometer using a dual target sample inlet probe.

NMR spectra of the intact peptide were recorded on a Bruker AM400 NMR spectrometer equipped with an ASPECT 3000 computer. All two-dimensional homonuclear spectra were recorded in the pure-phase absorption mode. The HOHAHA spectra were recorded with mixing times of 80 ms in D_2O, 74 ms in 90% H_2O/D_2O, and 160 ms in DMSO-d_6. The NOESY spectra were measured with mixing times of 300 ms in D_2O and in 90% H_2O/D_2O and 300 ms, 440 ms, and 660 ms in DMSO-d_6. A mixing time of 180 ms was set in the ROESY experiment in DMSO-d_6. For measurements in 90% H_2O/D_2O, the H_2O peak was suppressed by irradiation during the relaxation delay and in the case of NOESY spectra during the mixing time as well. HMBC and HMQC spectra were recorded essentially as described in the literature.[27]

Extraction and Isolation of Theonellamide F. The sponge (15 kg) was collected by hand using scuba at Hachijo Island at depths ranging between 10 and 20 m. The animal was frozen immediately after collection and kept frozen (−20 °C) until extraction. The sponge was homogenized with EtOH and extracted with EtOH (18 L), MeOH (3 × 15 L), and 50% aqueous *n*-PrOH (3 × 10 L). Combined EtOH and MeOH extracts were concentrated to an aqueous suspension (6 L) which was extracted with ether (2 × 2 L). The aqueous layer was centrifuged, and the supernatant was applied to a column of TSK G3000S (11 × 10 cm) and eluted successively with 2 L each of water, 40% MeOH, 60% MeOH, 80% MeOH, and MeOH, 1 L of $CHCl_3/MeOH/H_2O$ (70:30:5), and 2 L of $CHCl_3/MeOH/H_2O/AcOH$ (65:35:6:2). The precipitate obtained by centrifugation was dissolved in 50% aqueous DMSO and fractionated in the same manner. Antifungal activity against *Mortierella ramanianus* was found in the MeOH and $CHCl_3/MeOH/H_2O$ eluates. Combined active fractions and the aqueous *n*-PrOH extract (10 g in toto) were further separated on a column of silica gel (Yamamura Chem. Lab.; 250/350 mesh, 6 × 40 cm) by stepwise elution with $CHCl_3/MeOH/H_2O$ mixtures [70:30:5 (5 × 2 L), 70:32:5 (13 × 2 L), 68:33:5 (8 × 2 L), 65:35:8 (3 × 2 L), 6:4:1 (2 × 2 L)], MeOH (2 × 2 L), and 5% AcOH in MeOH (3 L). Each fraction was monitored by HPLC (YMC-ODS, AM-type, 35% *n*-PrOH in 20 mM KH_2PO_4 buffer, UV 290 nm), and fractions having the same retention times were collected. Fractions 9–14 (1.4 g) were mostly composed of theonellamide F. Further purification of portions was done by HPLC on ODS (YMC, AM-type, 2 × 25 cm, 32% *n*-PrOH in 20 mM KH_2PO_4) to yield 500 mg of **1**.

Theonellamide F (**1**): $[\alpha]^{23}_D$ −5.5° (*c* 0.12, 50% aqueous *n*-PrOH); IR (Nujol) 3300, 1650, 1540 cm^{-1}; ^1H NMR and ^{13}C NMR see Table I.

Acid Hydrolysis of Theonellamide F. For amino acid analysis, 100 μg of theonellamide F was dissolved in 0.5 mL of 6 N HCl in an evacuated glass tube and heated at 110 °C for 16 h. After evaporation, the residue was dissolved in 0.4 mL of 0.12 N HCl and subjected to amino acid analysis on a Hitachi 835 amino acid analyzer under conditions for physiological amino acids, in which Thr and *a*Thr were not separated. The presence of *a*Thr in the acid hydrolysate was verified by chiral GC–MS analysis. Retention times in the amino acid analysis (minutes): X-1 (10.8), Asp (18.6), X-2a (19.8), X-2b (24.1), *a*Thr (25.3), Ser (27.3), Phe (109.3), X-2b-lactone (111.2), X-2a-lactone (112.8), βAla (114.6), NH_3 (139.5), X-3 (150.3), X-4 (157.7). The peak area of X-3 was twice as large as that of Asp.

For a large-scale hydrolysis, a 50-mg portion of theonellamide F dissolved in 20 mL of 6 N HCl was heated at 110 °C for 16 h. After evaporation, the residue was dissolved in 2.5 mL of 0.05N Et_3N/HCO_2H buffer (pH 3.41) and subjected to HPLC on a column of Hitachi Custom Ion Exchange Resin 2614 (1 × 100 cm, flow rate 2 mL/min, detection by UV 215 nm and by refractive index). After elution with buffer for 150 min, the eluant was changed to the second buffer (0.5N Et_3N/AcOH, pH 5.10): retention time of amino acids (minutes), X-1 (49), Asp (93), *a*Thr (117), Ser (138), Phe (191), X-3 (194), βAla (203), X-2-lactones (214), BrPhe (240).

X-1: $[\alpha]^{23}_D$ +29.6° (*c* 0.1, 1 N HCl); ^1H NMR (D_2O) δ 4.21 (1 H, d, *J* = 3 Hz), 3.91 (1 H, d, *J* = 3 Hz); ^{13}C NMR (D_2O) δ 174.1 (s), 169.7 (s), 69.7 (d), 56.6 (d).

X-2a and X-2b (2:1 mixture): ^1H NMR (D_2O) δ 3.98 (1 H, quint, *J* = 6 Hz), 3.78 (1 H, t, *J* = 6 Hz), 2.30 (2 H, d, *J* = 6 Hz), 1.96 (2 H, t, *J* = 6 Hz); ^{13}C NMR (D_2O) δ 178.9 (s), 174.8 (s), 66.6 (68.4) (d), 53.3 (54.6) (d), 44.5 (t), 36.6 (37.2) (t). (Chemical shifts for the minor isomer, i.e., X-2b, are in parentheses.)

X-3: $[\alpha]^{23}_D$ +9.8° (*c* 0.07, 1 N HCl); ^{13}C NMR (D_2O) δ 174.4 (s), 172.0 (s), 139.3 (d), 136.4 (s), 119.1 (d), 55.6 (d), 55.4 (d), 47.6 (t), 29.3 (t); ^1H NMR (D_2O) δ 7.71 (7.72) (0.6 H, d, *J* = 1.1 Hz), 7.09 (1 H, br s), 4.56 (2 H, d, *J* = 5.1 Hz), 4.16 (4.15) (1 H, t, *J* = 5.1 Hz), 4.01 (4.00) (1 H, dd, *J* = 4.4, 7.5 Hz), 3.19 (3.20) (1 H, dd, *J* = 4.4, 15.5 Hz), 3.11 (3.08) (1 H, dd, *J* = 7.5, 15.5 Hz). (Chemical shifts for the minor isomer in parentheses.)

X-4: $[\alpha]^{23}_D$ −5.5° (*c* 0.08, H_2O); ^1H NMR (D_2O) δ 7.37 (2 H, d, *J* = 8.3 Hz), 7.01 (2 H, d, *J* = 8.3 Hz), 3.77 (1 H, dd, *J* = 5.6, 7.8 Hz), 2.96 (2 H, ABm).

Chiral GC–MS Analysis. Chrial GC–MS was carried out by using a Chirasil Val III capillary column (0.32 mm × 25 m) and JEOL DX303 mass spectrometer operating in the positive EI mode (scan range between *m/z* 50 and 600 with repetition time of 2 s). Derivatization of amino acid residues was done as reported in the literature[36] with slight modi-

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Table I. ^1H and ^{13}C NMR Signal Assignments of Theonellamide F

assignment		^{13}C[a] DMSO-d_6	^1H DMSO-d_6	^1H 90% H_2O
Ser(I)	α	55.2 (d)	4.12	4.37
	β	60.6 (t)	3.62, 3.62	4.27, 4.37
	NH		7.62	8.28
Aboa	α	36.4 (t)	2.25, 2.35	2.62, 2.99
	β	51.8 (d)	4.19	4.60
	γ	67.8 (d)	4.28	4.75
	δ	133.3 (d)	5.39	5.28
	ε	134.5 (s)		
	ε-Me	12.9 (q)	1.74	1.99
	ξ	134.1 (d)	6.74	6.63
	η	125.7 (d)	6.52	6.47
	1	136.4 (s)		
	2,6	127.9 (d)	7.42	7.22
	3,5	131.2 (d)	7.51	7.48
	4	119.8 (s)		
	NH		7.49	8.15
Phe	α	53.9 (d)	4.52	5.18
	β	37.8 (t)	2.72, 2.95	3.13, 3.13
	1	137.0 (s)		
	2,6	128.8 (d)	7.20	7.40
	3,5	127.8 (d)	7.23	7.40
	4	126.0 (d)	7.17	7.28
	NH		7.91	8.60
Ser(II)	α	55.8 (d)	4.32	5.01
	β	61.0 (t)	3.57, 3.71	4.27, 4.35
	NH		8.35	8.72
*a*Thr	α	57.5 (d)	4.28	4.73
	β	67.7 (d)	3.76	3.97
	γ	20.5 (q)	1.08	1.17
	NH		7.52	8.43
sHis	α	53.9 (d)	4.48	5.12
	β	30.6 (t)	2.70, 2.80	3.32, 3.63
	2	136.9 (d)	7.25	7.45
	4	136.4 (s)		
	5	117.7 (d)	6.83	7.45
	NH		7.97	8.61
isoAhad	α	49.5 (d)	4.28	4.69
	β	37.5 (t)	1.82, 1.88	2.49, 2.49
	γ	64.7 (d)	3.96	4.48
	δ	43.1 (t)	1.92, 2.32	2.83, 2.83
	NH		7.67	8.02
βAla	α	34.1 (t)	2.32, 2.38	2.91, 3.01
	β	35.6 (t)	3.05, 3.55	3.52, 4.01
	NH		7.43	8.02
BrPhe	α	54.2 (d)	4.34	4.85
	β	35.9 (t)	2.90, 2.90	3.06, 3.38
	1	136.8 (s)		
	2,6	131.0 (d)	7.01	7.18
	3,5	130.6 (d)	7.34	7.35
	4	119.1 (s)		
	NH		8.33	9.23
OHAsn	α	54.2 (d)	5.03	5.70
	β	71.1 (d)	4.12	4.62
	NH		8.18	8.88
	NH_2		7.19	7.43, 8.72[b]
Asn	α	50.5 (d)	4.62	5.02
	β	37.0 (t)	2.38, 2.62	2.68, 2.99
	NH		7.85	8.05
	NH_2		[c]	7.47, 7.98[b]
sAla	α	51.6 (d)	4.85	5.68
	β	47.2 (t)	4.00, 4.44	4.73, 5.39
	NH		7.97	8.78

[a] Chemical shifts for the carbonyl carbons: δ 173.6, 172.9, 170.9 (2 C), 170.8, 170.6 (2 C), 170.5, 170.2, 170.1 (2 C), 169.5, 169.0 (2 C), 168.7. [b] Assignments for the two pairs of NH_2 signals are interchangeable. [c] Not assigned.

fication. Acid hydrolysate (200 μg) was heated in 10% HCl in MeOH (0.5 mL) at 100 °C for 30 min in a screw-capped test tube. After removal of the methanolic HCl in vacuo, CH_2Cl_2 (0.3 mL) and trifluoroacetic anhydride (0.3 mL) were added, and the mixture was kept at 100 °C for 5 min. The product was evaporated, dissolved in CH_2Cl_2, and subjected to the analysis. Retention times (minutes): βAla (4.5), D-Ser (5.8), D-*a*Thr (6.2), L-Ser (6.6), L-*a*Thr (6.9), D-Asp (9.9), L-Asp (10.3), D-Phe (16.1), L-Phe (16.7), X2b (19.3), X2a (20.7), D-Dpr (20.9), L-Dpr (21.3), D-BrPhe (24.5), L-BrPhe (24.9). EI mass spectrum of bis(TFA) dimethyl ester of X2a: *m/z* 397 (M^+, 1%), 365 (6), 338 (5), 282 (10), 252 (15), 224 (100), 192 (78), 182 (27), 138 (22), 111 (18), 100 (12), 78 (17), 69 (17), 59 (15). (The mass spectrum of the derivatized X2b was indistinguishable from that of X2a.) EI mass spectrum of TFA methyl ester of X4: *m/z* 352/354 (M^+, 9%), 293/295 (7), 240/242 (100), 209/211 (19), 169/171 (83), 102 (15), 90 (29), 69 (11).

Preparation of 2 and 3. To an 8-mg portion of a 2:1 mixture of X2a and X2b in 50% aqueous dioxane (1 mL) was added BocON (50 mg) and Et_3N (100 μL), and the resultant mixture was stirred at room temperature for 4 h. The reaction mixture was extracted with EtOAc (3 × 1 mL), and the EtOAc layer was washed with water (3 × 0.5 mL). Combined aqueous layers were acidified with 0.5 mL of AcOH and extracted with EtOAc. The EtOAc phase was evaporated to yield 9 mg of N-protected γ-lactones, which were converted to the methyl esters by treatment with CH_2N_2/ether (2 mL) in MeOH (1 mL). The product was purified by normal-phase HPLC (LiChrosorb Si 60-5, 2 × 30 cm; *n*-hexane/ether, 1:3) with 6-fold recycling to obtain **2** (1.1 mg) and **3** (0.3 mg).

2: CD_{max} (MeOH) 217 nm [θ] = +810°; ^1H NMR ($CDCl_3$) δ 5.07 (1 H, br d, NH), 4.81 (1 H, dddd, *J* = 5.4, 6.4, 10.5, 12.0 Hz, H-4), 4.41 (1 H, ddd, *J* = 6.4, 8.3, 12.0 Hz, H-2), 3.72 (3 H, s; OMe), 2.94 (1 H, ddd, *J* = 5.4, 8.3, 12.5 Hz, H-3), 2.77 (2 H, m, H_2-5), 1.91 (1 H, ddd, *J* = 10.5, 12.0, 12.5 Hz, H-3′), 1.44 (9 H, s, *t*-Bu).

3: ^1H NMR ($CDCl_3$) δ 5.01 (2 H, m, NH, H-4), 4.38 (1 H, ddd, *J* = 6.4, 9.8, 9.8 Hz, H-2), 3.71 (3 H, s, OMe), 2.73 (2 H, m, H_2-5), 2.50 (2 H, m, H_2-3), 1.45 (9 H, s, *t*-Bu).

Synthesis of 4 and 5. A 4:1 diastereomeric mixture of **4** and **5** were synthesized from DL-Boc-allylglycine by way of bromolactonization with *N*-bromosuccinimide as reported.[37] The mixture was separated by HPLC as in case of the separation of **2** and **3**, without recycling.

4: ^1H NMR ($CDCl_3$) δ 5.09 (1 H, br d, NH), 4.62 (1 H, dddd, *J* = 5.9, 5.9, 10.3, 10.8 Hz, H-4), 4.44 (1 H, ddd, *J* = 6.1, 8.8, 11.8 Hz, H-2), 3.56 (2 H, m, H_2-5), 2.92 (1 H, ddd, *J* = 5.9, 8.8, 12.5 Hz, H-3), 1.99 (1 H, ddd, *J* = 10.3, 11.8, 12.5 Hz, H-3′), 1.44 (9 H, s, *t*-Bu).

5: ^1H NMR ($CDCl_3$) δ 5.12 (1 H, br d, NH), 4.90 (1 H, m, H-4), 4.39 (1 H, ddd, *J* = 6.4, 9.6, 9.6 Hz, H-2), 3.55 (2 H, m, H_2-5), 2.53 (2 H, m, H_2-3), 1.44 (9 H, s, *t*-Bu).

$KMnO_4$/$NaIO_4$ Oxidation of X-3. To a 200-μg portion of X-3 in water was added a solution of $KMnO_4$ (40 μg) and $NaIO_4$ (2 mg) in water. After standing at room temperature for 5 min, toluene (0.5 mL) and *n*-PrOH (0.5 mL) were added and left at room temperature for another 5 min. The product was evaporated, derivatized for GC, and analyzed by chiral GC–MS.

Preparation of L- and DL-2,3-Diaminopropionic Acid. L- and DL-2,3-diaminopropionic acids were synthesized essentially as reported in the literature[38] except for the use of the Boc N-protecting group instead of the carbobenzyloxy group.

Hydrogenation of Theonellamide F. To a solution of theonellamide F (2 mg in 2 mL of H_2O) was added 5% Pd–C (water wet) and hydrogenated (1 atm) at room temperature for 16 h. The reaction mixture was filtered, evaporated, and analyzed by FAB mass spectrometry, which revealed the MH^+ ion at *m/z* 1497. A 500-μg portion of the product was hydrolyzed, derivatized, and subjected to GC–MS analysis as described above. There were two peaks with an intensity ratio of 5:1 at R_t 29.4 and 29.8 min showing the same mass spectra, neither of which was observed in the hydrolysate of theonellamide F. EI mass spectrum of the TFA methyl derivative of HX5: *m/z* 471 (M^+, 20%), 440 (8), 358 (2), 357 (2), 325 (6), 295 (8), 263 (7), 244 (31), 212 (18), 199 (14), 184 (19), 159 (23), 131 (18), 104 (25), 91 (100), 74 (14), 55 (13).

Isolation of 8. To a solution of theonellamide F (110 mg) in 50 mL of 50% aqueous *n*-PrOH was added 5% Pd–C (water wet, 370 mg) and the resultant mixture was hydrogenated (1 atm) at room temperature overnight. The reaction mixture was evaporated, redissolved in 20 mL of 6 N HCl, and heated at 110 °C for 16 h in sealed test tubes. The product was centrifuged, and the precipitate was extracted with 20 mL each of 1 N HCl/*n*-PrOH (1:1) and 1 N HCl/acetone (1:1). Combined extracts were evaporated and partitioned between water (5 mL) and EtOAc (3 × 5 mL). The EtOAc layer was purified by preparative TLC (Merck precoated TLC plate, silica gel 60, 20 × 20 cm, 1-mm thick; $CHCl_3$/MeOH, 98:2). Compound **8** was obtained as the major UV absorbing band (R_f 0.35, yield 4 mg).

8: ^1H NMR [$CDCl_3$/CD_3OD (4:1) with trace CF_3CO_2D] δ 7.05–7.20 (5 H, m, aromatic protons), 4.40 (1 H, m, H-4), 3.94 (1 H, m, H-3), 3.31 (1 H, dd, *J* = 2.5, 18.0 Hz, H-2), 2.96 (1 H, dd, *J* = 8.0, 18.0 Hz, H-2′), 2.64 (1 H, ddd, *J* = 5.5, 10.2, 13.5 Hz, H-8), 2.55 (1 H, ddd, *J* = 6.0, 10.5, 13.5 Hz, H-8′), 1.75 (2 H, m, H-5, H-6), 1.62 (1 H, m, H-7), 1.51 (1 H, m, H-7′), 1.37 (1 H, m, H-5′), 0.99 (3 H, d, *J* = 6.5 Hz, 6-CH_3).

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Assignment was made by COSY spectrum.

Preparation of 9. For the isolation of **9**, another EtOAc-soluble portion was prepared as described above starting from 50 mg of **1**. To the material in 1% Me_3N/water (6 mL) was added a 5-mL portion of 5% dinitrofluorobenzene in EtOH, and the resultant mixture stirred for 2 h at room temperature. After evaporation of the solvent, the residue was partitioned between 1 N HCl (1 mL) and ether (3 × 1 mL). The ether layer was subjected to the preparative silica gel TLC ($CHCl_3$/AcOH, 98:2) and a major yellow band (R_f 0.4) was obtained. The $CHCl_3$-soluble portion of the resulting yellow material was purified by HPLC [YMC silica gel (5 μm) 2 × 25 cm, $CHCl_3$, detection at 345 nm] to obtain **9** (2.4 mg).

9: ^{1}H NMR ($CDCl_3$) DNP unit δ 9.17 (1 H, d, J = 2.7 Hz), 8.33 (1 H, dd, J = 2.7, 9.6 Hz), 6.79 (1 H, d, J = 9.6 Hz); X5-lactone unit δ 8.80 (1 H, d, J = 7.1 Hz, NH), 7.1-7.25 (5 H, m, aromatic protons), 4.83 (1 H, ddd, J = 3.9, 5.1, 10.1 Hz, H-4), 4.40 (1 H, dddd, J = 3.2, 5.1, 6.9, 7.1 Hz, H-3), 3.04 (1 H, dd, J = 6.9, 17.5 Hz, H-2), 2.70 (1 H, ddd, J = 5.5, 9.5, 14.0 Hz, H-8), 2.64 (1 H, dd, J = 3.2, 17.5 Hz, H-2′), 2.56 (1 H, ddd, J = 6.5, 9.5, 14.0 Hz, H-8′), 1.95 (1 H, ddd, J = 4.5, 10.1, 14.0 Hz, H-5), 1.77 (1 H, m, H-6), 1.67 (2 H, m, H_2-7), 1.43 (1 H, ddd, J = 3.9, 8.7, 14.0 Hz, H-5′), 1.02 (3 H, d, J = 6.5 Hz, 6-CH_3). Qualitative difference NOE experiment: irr δ 8.80, enhanced δ 4.40 (w, weak), 2.64 (m, medium), 1.95 (s, strong), and 1.43 (m); irr δ 4.83, enhanced δ 4.40 (s), 3.04 (w), 1.95 (w), 1.43 (m), and 1.02 (s); irr δ 4.40, enhanced δ 4.83 (s), 3.04 (s), and 2.64 (w).

Preparation of 10. To a solution of **9** (2 mg) in 0.2 mL of MeOH was added a 1-mg portion of K_2CO_3, which was stirred at room temperature for 1 h. After addition of $CHCl_3$ (2 mL), the reaction mixture was applied to a short silica gel column (1.5 × 3 cm) and eluted with $CHCl_3$/MeOH/H_2O (85:15:2) to give 1.8 mg of **10**.

10: FABMS (diethanolamine) m/z 537 (MH + diethanolamine)$^+$; ^{1}H NMR (CD_3OD) δ 9.02 (1 H, d, J = 2.7 Hz), 8.22 (1 H, dd, J = 2.7, 9.6 Hz), 7.20 (1 H, d, J = 9.6 Hz), 7.1-7.3 (5 H, m), 4.05 (2 H, m), 2.61 (4 H, m), 1.2-1.8 (5 H, m), 0.94 (3 H, d, J = 6.0 Hz).

Hoffman-Type Degradation of Theonellamide F with [Bis(trifluoroacetoxy)iodo]benzene (BTI).[38] To a solution of theonellamide F (7 mg) in 50% aqueous MeCN (6 mL) was added BTI (60 mg) and pyridine (100 μL). The mixture was stirred at room temperature for 2 days, evaporated, and partitioned between water and EtOAc. A 500-μg portion of the material was subjected to amino acid analysis and chiral GC-MS analysis.

Preparation of Theonellamide F Methyl Ester. To a solution of theonellamide F (1 mg) in water (0.5 mL) was added ethereal CH_2N_2 (1 mL), and the resultant mixture was left at room temperature for 30 min. After evaporation the product was subjected to FAB mass spectrometry (thioglycerol) which gave the MH$^+$ ion at m/z 1663.

Preparation of Theonellamide F Pentaacetate. Theonellamide F dissolved in a 1:1 mixture of Ac_2O/pyridine (1 mL) was stirred at room temperature overnight. The product was dried and subjected to FAB mass spectrometry (thioglycerol) to give the MH$^+$ ion at m/z 1859.

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Synthesis and Absolute Configuration of the *Aristotelia* Alkaloid Peduncularine

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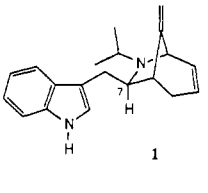
Abstract: The first synthesis of the *Aristotelia* alkaloid peduncularine (**1**) is described. The synthetic sequence commences with (*S*)-malic acid and amounts to 16 steps. Key transformations are (1) the stereoselective transalkylation of the β-hydroxylactam dianion derived from **8**, (2) the formation of the azabicyclo[3.2.1]octanone skeleton **6** via a silicon-assisted *N*-acyliminium ion cyclization of **7**, (3) the introduction of the endocyclic double bond in **5** by flash-vacuum thermolysis of an acetate, and (4) the four-step conversion of lactam **5** into the target molecule **1**. This work conclusively establishes the structure and absolute stereochemistry of natural peduncularine. In addition to **1**, the synthesis also furnishes 7-*epi*-peduncularine (**2**). Contrary to the conclusion in a recent publication, the structures of natural isopeduncularine and 7-*epi*-peduncularine are different.

Peduncularine is the principal alkaloid of the Tasmanian shrub *Aristotelia peduncularis* (Elaeocarpaceae).[1] Bick and co-workers reported the isolation of this natural product in 1971 and initially assigned to it an indole-pyrrolizidine structure on the basis of limited spectroscopic data.[2] Several years later,[3] the revised structure **1** was put forward, containing the unique 6-azabicyclo[3.2.1]-3-octene skeleton with a 3-indolylmethyl substituent. This structure for peduncularine was in complete accordance with



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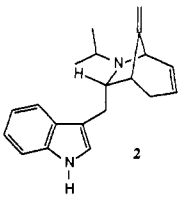

2

the results of extensive spectroscopic and degradative work.[3] Recently, a closely related alkaloid was reported to accompany **1** in *A. peduncularis*.[4] This new base, also isolated from *Aristotelia fruticosa*[4] and *Aristotelia serrata*,[5] small trees of New

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