Orbiculamide A: A Novel Cytotoxic Cyclic Peptide from a Marine Sponge *Theonella* sp.[1]

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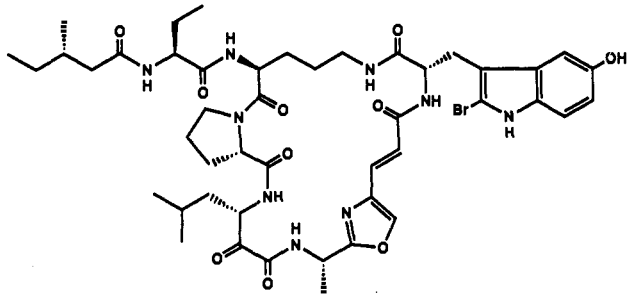
Marine sponges of the genus *Theonella* often afford bioactive cyclic peptides containing unusual amino acid residues, e.g. theonellamide F,[2] cyclotheonamides,[3] and theonellapeptolides.[4] We now report orbiculamide A from the same marine sponge, *Theonella* sp., that contained cyclotheonamides, potent antithrombin cyclic peptides. Orbiculamide A is cytotoxic against P388 murine leukemia cells (IC_{50} 4.7 μg/mL).

Theonella sp.[5] (10.5 kg) collected by SCUBA was extracted with EtOH; the extracts were partitioned between H_2O and Et_2O. The organic phase (70.9 g) was further partitioned between *n*-hexane and MeOH/H_2O (9:1). The aqueous MeOH layer was fractionated by flash chromatography on ODS with aqueous MeOH, followed by gel filtration on Sephadex LH-20. The active fractions were purified by centrifugal counter-current chromatography with hexane–EtOAc–MeOH–H_2O (3:7:5:5) and finally by reverse-phase HPLC (ODS, 60–76% MeOH–H_2O with 0.05% TFA) to yield 39.2 mg of orbiculamide A as a colorless powder (3.7×10^{-4}% yield based on wet weight).[6]



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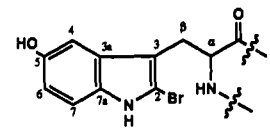
Orbiculamide A (**1**) had a molecular formula of $C_{46}H_{62}BrN_9O_{10}$, which was established by a combination of FABMS and NMR data.[7] Since its peptide nature was evident from the ^1H and ^{13}C NMR spectra, the compound was subjected to standard amino acid analysis, which revealed the presence of one residue each of Pro, Ala, 2-aminobutyric acid (Aba), and Orn. In addition to these amino acid residues, three new amino acid residues, **2**–**4**,



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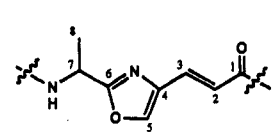

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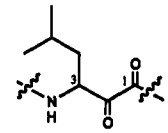

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as well as 3-methylvaleric acid were present on the basis of evidence provided by 2D NMR spectral data, including COSY, HOHAHA,[8] ROESY,[9] HMQC,[10] and HMBC[11] in DMSO-d_6 and in CD_3OH. 2-Bromo-5-hydroxytryptophan (Bhtrp, **2**) was inferred from the UV (λ_{max} 203, 220, 269, 303 nm)[12] and ^1H and ^{13}C NMR spectra,[13] which are fully consistent with the HMBC data. The second new amino acid, named theonalanine (Thl, **3**), showed a sharp singlet at δ 7.92 (H5), which was not only coupled to C5 (δ 140.4) by 207 Hz but also showed HMBC correlations with C4 (δ 138.7) and C6 (δ 165.7). These chemical shifts and the $^1J_{CH}$ value of C5 were reminiscent of an oxazole ring.[14] Further HMBC correlation (C6/H7, Me8; C5/H3; C4/H2, H3; C1/H2, H3) allowed us to assign the gross structure **3**. Incidentally, theonalanine liberated Ala upon acid hydrolysis.[15] Though undetectable in the amino acid analysis, there was a ^1H NMR spin system assignable to a Leu residue, in which a nitrogen-bearing methine proton (δ 5.12, H3) exhibited an HMBC cross peak with a C2 ketone signal at δ 195.9. This carbon could be placed adjacent to an amide carbon at δ 160.9, which is reminiscent of an α-keto amide, as in the case of cyclotheonamides.[3] Thus, the remaining amino acid, now named theoleucine (Tle), was **4**. Assignment of the NMR signals for Pro, Aba, Orn, and 3-methylvaleryl residues was unexceptional.

(1) Part 36 of the Bioactive Marine Metabolites Series. Part 35: Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Org. Chem.* **1991**, *56*, 4971–4974.

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(3) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, H. *J. Am. Chem. Soc.* **1990**, *112*, 7053–7054.

(4) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Tetrahedron* **1991**, *47*, 2169–2180.

(5) The sponge was collected off Hachijo-jima Island at −15 to −20 m. While collecting the sponge, we were entertained by a small school of the batfish, *Platax orbicularis*, from which the name of the compound was coined.

(6) Orbiculamide A gave a broad peak in HPLC, which made large scale purification difficult. The sample which we used for NMR experiments contained 10–20% of a minor component, orbiculamide B, which has an identical cyclic nucleus, except for the replacement of the methyl on C7 by an ethyl in residue 3. The structures of minor components will be reported elsewhere.

(7) **1**: $[\alpha]^{23}_D$ −60° (*c* 0.005, MeOH); UV (MeOH) 203 (ε 14 100), 220 (ε 10 200), 269 (ε 6900), 303 (ε 1500) nm; FABMS (negative, glycerol matrix) *m/z* 980, 978 (1:1, M − H^-), 900 (M − Br^-); ^{13}C NMR data in CD_3OH at 313 K: Pro residue 174.9 (CO), 60.6 (α), 30.5 (β) 25.8 (γ), 48.8 (δ); Aba residue 174.0 (CO), 55.9 (α), 26.1 (β), 10.6 (γ); Orn residue 172.1 (CO), 52.3 (α), 30.2 (β), 26.2 (γ), 40.3 (δ); Bhtrp residue 173.3 (CO), 53.5 (α), 27.9 (β), 112.3 (C2), 109.3 (C3), 129.7 (C3a), 104.7 (C4), 151.9 (C5) 112.8 (C6), 111.9 (C7), 132.9 (C7a); Thl residue 168.1 (C1), 124.9 (C2), 129.1 (C3), 138.7 (C4), 140.4 (C5), 165.7 (C6), 46.0 (C7), 17.8 (C8); Tle residue 160.9 (C1), 195.9 (C2), 54.9 (C3), 39.0 (C4), 26.5 (C5), 23.5 (C6), 21.1 (C7); 3Mv residue 175.7 (C1), 44.1 (C2), 33.5 (C3), 30.4 (C4), 11.5 (C5), 19.3 (C6); ^1H NMR data in CD_3OH at 313 K: Pro residue 4.44 (dd, 8.3, 5.3; α), 1.93 (m; β), 2.28 (m; β′), 1.82 (m; γ), 1.95 (m; γ′), 2.83 (m; δ), 3.61 (m; δ′); Aba residue 4.24 (m; α), 1.61 (m; β), 1.77 (m; β′), 0.92 (3H, t, 7; γ), 7.92 (m; NH); Orn residue 4.36 (m; α), 1.39 (m; β), 1.46 (m; β′), 1.27 (m; γ), 1.42 (m; γ′), 2.61 (m; δ), 3.75 (m; δ′), 7.92 (m; αNH), 7.51 (m; δNH); Bhtrp residue 4.96 (m; α), 3.11 (dd, 14.5, 6.3; β), 3.45 (dd, 14.5, 3.1; β′), 6.97 (d, 1.6; H4), 6.63 (dd, 8.5, 1.6; H6), 7.06 (d, 8.5; H7), 7.81 (d, 9.7; αNH), 10.85 (br s; 1NH); Thl residue 6.92 (d, 15.2; H2), 7.28 (d, 15.2; H3), 7.92 (s; H5), 4.97 (m; H7), 1.57 (3 H, d, 7.2; Me8), 8.84 (d, 7.2; NH); Tle residue 5.12 (m; H3), 1.38 (m; H4), 1.68 (m; H4′), 1.85 (m; H5), 0.94 (3 H, d, 6.7; Me6), 0.94 (3 H, d, 6.7; Me7), 8.35 (d, 5.8; NH); 3Mv residue 2.03 (dd, 13.8, 8.6; H2), 2.24 (dd, 13.8, 5.9; H2′), 1.85 (m; H3), 1.23 (m; H4), 1.36 (m; H4′), 0.90 (3 H, t, 7; Me5), 0.91 (3 H, d, 7; Me6).

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The sequencing of the seven segments was done by an HMBC experiment in CD_3OH, which provided correlations through all amide bonds, except for the bond between Orn and Pro. However, the connectivity through the prolyl nitrogen and the carboxyl group of the Orn residue was implied by both the ROSEY spectrum recorded in CD_3OH and NOESY data measured in DMSO-d_6, which also supported the entire sequence.

The configurations of Ala (C7 of **3**), Aba, and Orn residues were determined to be L by chiral GC on a Chirasil Val III column (Alltech). Assignment of L-Pro was accomplished by HPLC after derivatization with Marfey's reagent.[16] Treatment of orbiculamide A with $NaIO_4/KMnO_4$, followed by acid hydrolysis, yielded L-Asp, as revealed by chiral GC analysis; thus **2** has L configuration. Oxidation of **1** with H_2O_2/aqueous NaOH[17] followed by acid hydrolysis afforded L-Leu which was detected by chiral GCMS, thereby establishing 3*S* stereochemistry of **4**. The lipophilic portion of the acid hydrolysate was converted to the (*S*)-1-naphthylethylamide[18] and analyzed by GC (OV-1), which revealed *S* stereochemistry of the 3-methylvaleric acid residue.

Orbiculamide A is another example of a cyclic peptide[19] containing three new amino acids, 2-bromo-5-hydroxytryptophan (**2**), theonalanine (**3**), and theoleucine (**4**). α-Keto β-amino acids appear to be a characteristic feature of peptides from sponges of the genus *Theonella*.[3]

Acknowledgment. We thank Professor Paul J. Scheuer, University of Hawaii, for reading this manuscript. Thanks are also due to Dr. Y. Numazaki and Ms. C. Nohara of the Central Research Laboratories of Yamanouchi Pharmaceutical Co., Ltd. for cytotoxicity tests and to Professor K. Mori, University of Tokyo, for a generous gift of an authentic sample of 3-methylvaleric acid. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Supplementary Material Available: ^1H NMR spectrum in DMSO-d_6 and ^{13}C NMR, HOHAHA, ROESY, HMQC, and HMBC spectra in CD_3OH for **1** (9 pages). Ordering information is given on any current masthead page.

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(19) J. Kobayashi and co-workers have also isolated peptides closely related to our compound from an Okinawan *Theonella* sponge as shown in an accompanying paper. We are indebted to Professor J. Kobayashi for ^1H and ^{13}C NMR and FABMS data.

Keramamides B-D: Novel Peptides from the Okinawan Marine Sponge *Theonella* sp.

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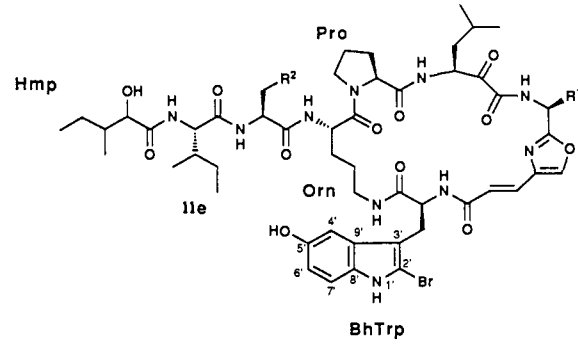
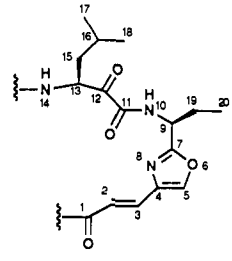
Marine sponges of the genus *Theonella* have been demonstrated to be a rich source of bioactive secondary metabolites with unique chemical structures.[2] During our investigations on bioactive substances from Okinawan marine organisms,[3] we isolated three novel peptides, keramamides B-D (**1-3**), from a sponge *Theonella* sp. Here we describe the isolation and structure elucidation of **1-3**.



1 R^1=CH_2CH_3, R^2=CH_2CH_3
2 R^1=CH_2CH_3, R^2=CH_3
3 R^1=CH_3, R^2=CH_3

The methanol/toluene (3:1) extract of the sponge, collected off Kerama Islands, Okinawa, was partitioned between toluene and water. The chloroform extract of the aqueous phase was subjected to flash chromatography on a silica gel column with methanol/chloroform (15:85) followed by gel filtration on Sephadex LH-20 with methanol and reversed-phase HPLC on ODS (methanol/water/trifluoroacetic acid, 70/30/0.1; 2.0 mL/min) to give keramamides B[4] (**1**; 0.00017% yield, wet weight), C (**2**; 0.00027%), and D (**3**; 0.00023%).

The molecular formula of keramamide B (**1**) was established to be $C_{54}H_{77}O_{12}N_{10}Br$ by HRFABMS data [*m/z* 1137.5000 (M + H)$^+$ for $C_{54}H_{78}O_{12}N_{10}Br$, Δ +1.5 mmu]. Though the ^1H NMR spectrum suggested **1** to be a peptide, **1** was negative to ninhydrin and positive to Fast Red B salt,[5] implying the absence of an N-terminus and the presence of a tryptophan derivative. The standard amino acid analysis of the hydrolysate of **1** showed the presence of 1 mol each of proline (Pro), ornithine (Orn), isoleucine (Ile), α-aminobutyric acid (Aba), and norvaline (nVal). Extensive analysis of the ^1H and ^{13}C NMR data of **1**[6] including ^1H-^1H COSY, HOHAHA,[7] HMQC,[8] and HMBC[9] spectra revealed the presence of 2-bromo-5-hydroxytryptophan (BhTrp) and partial structure **4**. For the BhTrp residue the ^1H and ^{13}C signals were



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firmly assigned by the ^1H-^{13}C long-range connectivities observed through the HMBC spectrum and the ^1H and ^{13}C chemical shifts were consistent with those of 2-bromo-[10] and 5-hydroxyindole[11] derivatives. Segment **4** was deduced by the NMR data to consist

(1) (a) Hokkaido University. (b) Sankyo Co., Ltd. (c) The University of Tokyo. (d) Tohoku University.
(2) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053-7054 and references cited therein.
(3) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574-4576 and references cited therein.
(4) **1**: $[\alpha]^{23}_D$ -50° (*c* 0.7, MeOH); IR (KBr) ν_{max} 3390, 1660, 1640, 1530, 1460, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 267 (ε 23 500) and 312 (4300) nm.
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