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]

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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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(17) Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952**, *17*, 141-148. Partially racemized Leu was obtained when 35% H_2O_2/1 N NaOH was employed, whereas L-Leu was exclusively detected upon treatment with 35% H_2O_2/0.1 N NaOH.
(18) Bergot, B. J.; Anderson, R. J.; Schooley, D. A.; Henrick, C. A. *J. Chromatogr.* **1978**, *155*, 97-105.
(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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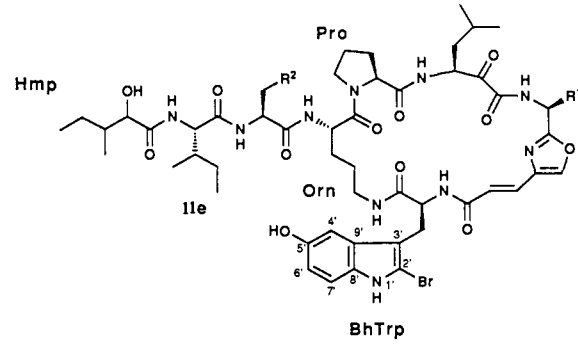
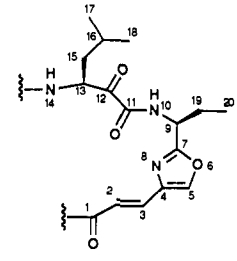
Marine sponges of the genus *Theonella* have been demonstrated to be a rich source of bioactive secondary metabolites with unique chemical structures.[2] During our investigations on bioactive substances from Okinawan marine organisms,[3] we isolated three novel peptides, keramamides B-D (**1-3**), from 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



4

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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of three modified amino acid residues containing an α,β-unsaturated amide group conjugated with an oxazole[12] as well as an α-keto-β-amino acid moiety constituting a homoleucine-like unit. Interpretation of the HMBC and NOESY spectral data provided evidences for the amino acid sequence of **1**. As a result, **1** was deduced to be composed of a cyclic moiety and a side chain. The cyclic moiety consisted of segment **4**, Pro, Orn, and BhTrp connected in this sequence. The side chain was attached to the α-NH of the Orn residue and consisted of nVal and Ile. The N-terminus of Ile was shown to be protected by the 2-hydroxy-3-methylpentanoic acid group (Hmp).[13] Further substantial evidence for the structure of keramamide B (**1**) was obtained by the FAB MS/MS[14] spectrum of the $(M + H)^+$ ion of **1** (m/z 1137), which afforded daughter ions corroborating well the amino acid sequence deduced from the NMR results. The chiral GC analysis (Chirasil-Val, Alltech) of the *N*-trifluoroacetyl/methyl ester derivatives of the hydrolysate of **1** revealed that all of the Aba,[15] Pro, Orn, nVal, and Ile residues of **1** bore the L configurations. The BhTrp residue in **1** was converted into aspartic acid (Asp) by ozonolysis of **1** followed by oxidation with CH_3CO_3H, while the C-11–N-14 moiety in segment **4** was transformed into leucine (Leu) by treatment with H_2O_2/NaOH. These Asp and Leu residues were determined to be L by the chiral GC method, revealing that the BhTrp and the β-amino amino acid in **4** were also L. Therefore, the structure of keramamide B was established to be that of **1**.

Molecular formulas of keramamides C (**2**) and D (**3**) were determined to be $C_{53}H_{75}O_{12}N_{10}Br$ and $C_{52}H_{73}O_{12}N_{10}Br$, respectively, by HRFABMS data. The amino acid analyses and FAB MS/MS results revealed that keramamide C (**2**) contained an Aba residue in place of the nVal residue in keramamide B (**1**). For keramamide D (**3**) the nVal in **1** was replaced by an Aba residue and the ethyl group attached to C-9 of segment **4** was substituted by a methyl group.[16]

Keramamides B–D (**1–3**; 5×10^{-8} M) inhibited the superoxide generation response of the human neutrophils[17] elicited with a chemotactic peptide, *N*-formyl-Met-Leu-Phe (fMLP), but did not inhibit that induced by phorbolmyristate acetate or immune complex.[18] The mechanism of action is currently under investigation.[19]

Acknowledgment. We thank Mr. Z. Nagahama for his help in collecting the sponge and Professor T. Sasaki, Kanazawa University, for the cytotoxicity test.

Supplementary Material Available: Notes for spectral data and spectra of keramamides B–D (36 pages). Ordering information is given on any current masthead page.

(6) **1**: ^{1}H NMR (DMSO-d_6) [**4**] 6.58 (d, 15.0; 2), 7.15 (d, 15.0; 3), 8.21 (s; 5), 4.63 (m; 9), 9.04 (d, 5.6; NH-10), 4.89 (m; 13), 8.15 (d, 5.1; NH-14), [BhTrp] 8.08 (d, 10.0; NH), 4.60 (m; α), 2.85 (dd, 14.5, 2.8; β), 3.06 (dd, 14.5, 5.5; β'), 11.21 (s; NH-1'), 6.88 (d, 2; 4), 6.58 (dd, 8.7, 2; 6), 7.03 (d, 8.7, 7), 8.64 (s; OH-5), [Pro] 4.35 (br d, 3.9; α), 1.85 (m; β), 2.15 (m; β'), 3.51 (m; δ), 3.74 (m; δ'), [Orn] 7.83 (d, 7.8; α-NH), 4.49 (m; α), 3.35 (m; δ), 2.65 (m; δ'), 7.43 (m; δ-NH), [nVal] 8.05 (d, 8.1; NH), 4.25 (m; α), [Ile] 7.50 (d, 9.3; NH), 4.28 (m; α), 1.70 (m; β), [Hmp] 3.74 (m; α), 5.45 (m; OH-α), 1.70 (m; β), 0.90 (m; CH_3-γ), 1.15 (m; CH_2-γ), and 0.90 (m; CH_3-δ); ^{13}C NMR (DMSO-d_6) [**4**] 163.9 (1), 123.5 (2), 127.4 (3), 139.6 (4), 136.9 (5), 164.8 (7), 50.0 (9), 160.1 (11), 195.8 (12), 52.5 (13), 39.0 (15), 24.8 (16), 20.8 (17), 23.2 (18), 25.2 (19), 10.5 (20), [BhTrp] 170.7 (CO), 53.4 (α), 27.9 (β), 109.5 (2'), 109.0 (3'), 102.3 (4'), 150.8 (5'), 111.5 (6'), 110.9 (7'), 130.5 (8'), 128.3 (9'), [Pro] 170.6 (CO), 58.4 (α), 29.6 (β), 23.2 (γ), 46.9 (δ), [Orn] 49.7 (α), 28.6 (β), 24.4 (γ), 38.9 (δ), [nVal] 171.2 (CO), 52.1 (α), 34.0 (β), 18.5 (γ), 13.6 (δ), [Ile] 169.4 (CO), 53.8 (α), 37.3 (β), 24.7 (γ), 15.4 (Me), 11.0 (Me'), [Hmp] 172.6 (CO), 75.0 (α), 38.2 (β), 24.2 (γ), 15.5 (Me), and 11.7 (Me').

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(11) Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699–7702.
(12) The $^1J_{CH}$ value between C-5 and H-5 was determined to be 209.6 Hz by the INEPT experiment: Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* 1979, *101*, 760–762.
(13) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236–241.
(14) McLafferty, F. W. *Science* **1981**, *214*, 280–287.
(15) The Aba residue was derived from the C-7–N-10 moiety in segment **4**.
(16) Keramamides B–D (**1–3**) were shown to possess closely related structures to that of a peptide recently isolated by Professor N. Fusetani and his co-workers. The ^{1}H NMR and MS spectral data were compared with each other before submission of this manuscript.
(17) Shirato, M.; Takahashi, K.; Nagasawa, S.; Koyama, J. *FEBS Lett.* **1988**, *234*, 231–234.
(18) Human neutrophils are known to generate superoxide anion, when exposed to appropriate stimuli, such as phorbolmyristate acetate, ovalbumin complex of immunoglobulin G_2 antibody, and fMLP. Therefore the present results suggest that a certain factor inhibited by keramamides B–D (**1–3**) is involved in the intracellular signal transduction processes initiated by fMLP.
(19) Keramamides B–D (**1–3**) exhibited no cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro at 10 μg/mL.

New Synthesis of Methyl 1,3-Butadiene-2-carboxylate by the Cheletropic Extrusion of Carbon Monoxide from 3-Carbomethoxy-3,4-pentadienal and a Study of Its Dimerization To Give Dimethyl Mikanecate (Dimethyl 4-Ethenyl-1-cyclohexene-1,4-dicarboxylate)

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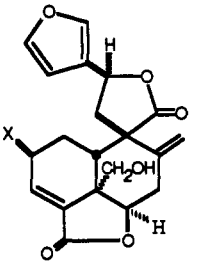
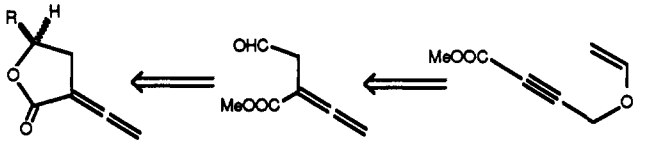
For a synthetic approach to plaunol B and C (**1a,b**), diterpenoids isolated from *Croton sublyratus* Kurz which show anti peptic ulcer activity,[1] we required an easy preparation of the 5-(3-furyl) allenic lactone **2a**. A reasonably short route would involve the thermal [3,3]-sigmatropic rearrangement[2] of the readily available 3-carbomethoxypropynyl ethenyl ether **3** to give the allenic acetaldehyde **4** followed by addition of an aryl anion and subsequent lactone formation. We report here the discovery of a novel thermal rearrangement of 3-carbomethoxy-3,4-pentadienal (**4**) to produce methyl 1,3-butadiene-2-carboxylate (**5**). In addition, we present the kinetic parameters for the very facile dimerization of **5** via a Diels–Alder cycloaddition.[3]



1a X = H
1b X = OH



2a R = 3-furyl
2b R = Ph

4 **3**

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(2) (a) Black, D. K.; Landor, S. R. *J. Chem. Soc.* **1965**, 6784. (b) Black, D. K.; Fomum, Z. T.; Landor, P. D.; Landor, S. R. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1349. (c) For a good recent review of allene rearrangements, see: Braverman, S. Rearrangements involving Allenes. In *The Chemistry of Double-bonded Functional Groups*; Patai, S., Ed.; Wiley: New York, 1989; Chapter 14.

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