methanol almost did not affect the ratio of the rates. The O–O bond cleavage of hydroperoxides almost stoichiometrically afforded 1 equiv of the alcohols and 2 equiv of DPP• or TBP• in the reactions. This oxidation process consists of the formation step of the alkyl peroxide–iron porphyrin complex, the O–O bond cleavage step, and the reaction step of the reactive intermediates and the substrates. Thus, the acceleration of the reaction by thiolate ligation is undoubtedly due to the enhancement of O–O bond scission and/or the first step since the third step is known to be very fast.[13,14] It is highly probable that the acceleration of O–O bond cleavage dominates because the high concentration of the used peroxides makes the cleavage step rate determining. In an interpretation of the thiolate ligand effect on the reaction of P-450, Dawson et al. have proposed that large electron donation to the iron from the thiolate enhances cleavage of the O–O bond.[12] A cyclic voltammogram of **1** in DMF showed a clear, reversible reduction couple (Fe(III)/Fe(II)) at −0.45 V vs SCE, which is more negative than that of FeTPP(III)Cl (−0.27 V vs SCE). The negativity of the redox potential of **1** is probably due to the electron donation of thiolate to iron atom. Both our kinetic and electrochemical results can be considered to support the speculation by Dawson et al. experimentally.

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(16) These experiments indicate that the reactions of iron porphyrins with peroxide form active intermediates which can oxidize TBPH or DPPH, whether the axial ligand of the complex is thiolate or not. Groves et al. reported that alkanes and alkenes are readily oxygenated by oxoiron(IV) porphyrin π cation radical which is formed by the reaction of iodosylbenzene or *m*-CPBA with iron porphyrin of which the axial ligand is chloride.[17] It was difficult to prepare pure active species derived from **1** with peroxides in the absence of a substrate at ordinary temperature. To investigate the property of the active species is the subject of our next study.

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Cyclotheonamides, Potent Thrombin Inhibitors, from a Marine Sponge *Theonella* sp.[1]

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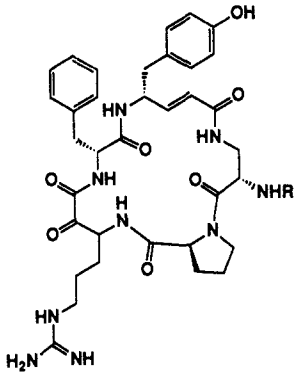
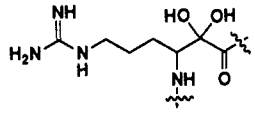
Recent chemical studies have shown that marine sponges of the genus *Theonella* are a rich source of secondary metabolites possessing potent bioactivities and novel chemical features; e.g., swinholide A,[2] bistheonellides,[3] onnamide A,[4] theonellamide F,[5] and theonellapeptolides.[6] In the course of our screening program of Japanese marine invertebrates for potential biomedicals,[7] we encountered a marine sponge of the genus *Theonella*[8] collected off Hachijo-jima Island, 300 km south of Tokyo, which strongly inhibited various proteinases, particularly thrombin. We have isolated two active substances, named cyclotheonamides A and B, which proved to be novel cyclic peptides. This communication deals with the isolation and structural elucidation of these peptides.

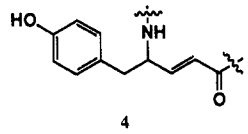
The concentrated ethanol extract of the sponge (10 kg wet weight) collected in 1987 was extracted with ether followed by 1-butanol (yield, 29.5 g). The 1-butanol phase was successively gel-filtered on Sephadex LH-20 and Toyopearl HW40 SF with methanol as eluent. The active fractions[9] were subjected to HPLC on Asahipak GS320 (aqueous CH_3CN) and on Senshu Pak ODS-H-4251 (first with 40–50% CH_3OH in 50 mM aqueous Na_2SO_4 and then with 10–30% CH_3CN in 0.1% TFA) to furnish cyclotheonamide A (**1**, 50 mg, (5×10^{-4})% yield).[10] The same sponge (4.5 kg wet weight) collected in 1989 from the same location yielded cyclotheonamide B (**2**, 4.6 mg, (1×10^{-4})% yield).[11]



1 R=CHO
2 R=Ac



3



4

The FAB mass spectrum of cyclotheonamide A in methanol solution gave an $(MH + CH_3OH)^+$ ion at *m/z* 764 as well as an $(MH)^+$ ion at *m/z* 732, whereas the highest ion peak shifted to *m/z* 778[12] $(MH + C_2H_5OH)^+$ when the sample was dissolved in ethanol. The negative FABMS gave an $(M - H)^-$ ion at *m/z* 730 confirming the molecular weight of 731.

Since the ^1H NMR spectrum inicated **1** to be a peptide, it was subjected to the standard amino acid analysis, which implied the presence of 1 mol each of Pro, Phe, and 2,3-diaminopropionic acid (Dpr).[13] Extensive NMR analysis on **1** including COSY, CH-COSY,[14] and HMBC[15] spectra in D_2O revealed the spin systems for two hitherto unknown amino acid residues, **3** and **4**, as well as those for Pro, Phe, and Dpr.[16]

(1) Bioactive Marine Metabolites. 33. Part 32: Hirota, Y.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1990**, *31*, 4763–4764.

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(7) Fusetani, N. Paper was presented at the Sixth International Symposium on Marine Natural Products at Dakar, 1989; to be published in *New J. Chem.*

(8) This sponge is characterized by a brilliant yellow inner body which is different from that of another *Theonella* sponge containing theonellamides.

(9) Inhibitory activity against thrombin was assayed according to Svendsen et al.: Svendsen, L.; Blomback, B.; Blomback, M.; Olsson, P. I. *Thromb. Res.* **1972**, *1*, 264–278.

(10) **1**: $[\alpha]^{23}_D$ −13° (*c* = 0.2, MeOH); UV (MeOH) 278 nm (ϵ 1940). IC_{50} (μg/mL): thrombin, 0.076; trypsin, 0.2; plasmin, 0.3. Full physicochemical as well as biological data for **2** will be reported in a forthcoming full account.

(11) The cyclotheonamide B preparation contained a small amount (ca. 5%) of **1**, while the cyclotheonamide A preparation was free of **2**.

(12) Precise ion matching gave a composition of $C_{38}H_{52}N_9O_9$ (*m/z* 778.4039, Δ 15.1 mmu).

(13) The amino acid analysis (ion exchange/ninhydrin) revealed the presence of one basic amino acid, probably **3**, which had a little shorter retention time than that of Arg in addition to three small peaks, probably degradation products of **4**, appearing near the retention time of Lys.

(14) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987.

(15) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.

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NMR data for **3** were identical with those of Arg except for a signal of the C-1 carboxyl carbon, while the α-proton[17] showed a correlation with an sp^3 carbon at δ 97.4 in the HMBC spectrum. This carbon appeared at δ 196.9 in DMSO-d_6, thus suggesting conversion from a hydrated to a simple carbonyl. Therefore, it is reasonable to place this carbon next to an amide carbon (δ 174.7) which showed no correlation peaks in the HMBC spectrum. Thus, this new amino acid has structure **3**. The structure of **4** was deduced from ^1H and ^{13}C NMR data. The presence of a formyl group (δ_H 8.08, δ_C 166.7 d) attached to the α-amino group of Dpr was revealed by the HMBC data. Connecting the five amino acids (Pro, Phe, Dpr, **3**, and **4**) by five amide bonds resulted in a molecular weight of 749, corresponding to the weight of the hydrated carbonyl, while the FABMS weight of 731 is that of the dehydrated carbonyl. Ready hemiketal formation explains the mass spectral data for the species with added alcoholic solvents.

Partial sequencing from the HMBC spectrum (D_2O) was deduced by cross peaks between the α-proton of **3** and the carbonyl of Pro, and between the β-protons of Dpr and the carbonyl of **4**, but no further information was obtained from NMR spectra measured in D_2O. Since NMR spectra recorded in DMSO[18] revealed well-resolved signals, final sequencing was determined from the NOESY spectrum,[19] which led to gross structure **1**.

To our surprise, cyclotheonamide B (**2**) obtained from the 1989 collection lacked the formyl proton in the ^1H NMR spectrum, which was replaced by a sharp methyl singlet at δ 2.00, indicating the presence of an *N*-acetyl Dpr residue. From our experience with **1**, we decided to measure NMR spectra of **2** in 90% H_2O–D_2O solution, which afforded valuable information. The COSY and the TOCSY (HOHAHA)[20] spectra of **2** revealed the same amino acid residues as in **1**. An HMBC spectrum disclosed the crucial amide protons and hence all connections of the five residues.[21] Though the NOESY spectrum in 90% H_2O disclosed merely sequential information, the ROESY spectrum[22] yielded a number of informative cross peaks, which allowed us to confirm the sequential assignment obtained by the HMBC spectrum.

The stereochemistries of Pro, Phe, and Dpr were determined to be L, D, and L, respectively, by chiral GC on a Chirasil Val III (Alltech) column.[23] The configuration of **4** was determined by degradation of **1** with $KMnO_4$–$NaIO_4$ followed by chiral GC analysis, which resulted in D-Asp, thus suggesting the 4*R* stereochemistry of **4**. Incidentally, Asp is not released from Phe by these conditions. Elucidation of the stereochemistry of **3** is in progress.

The amino acids **3** and **4** appear to be new.[24] β and γ amino acid residues seem to be characteristic of highly bioactive peptides from marine organisms.[25]

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Supplementary Material Available: HMBC and NOESY spectra for **1** and HMBC, TOCSY, and ROESY spectra for **2** (5 pages). Ordering information is given on any current masthead page.

(16) ^{13}C NMR data for **1** in D_2O: Phe residue 173.8 (CO), 57.0 (α), 41.8 (β), 138.2 (1), 132.2 (2, 6), 131.3 (3, 5), 130.0 (4); **4** residue 170.5 (CO), 125.5 (α), 146.3 (β), 55.5 (γ), 40.5 (δ), 132.4 (1), 133.4 (2, 6), 118.5 (3, 5), 157.4 (4); Dpr residue 173.1 (CO), 51.7 (α), 42.3 (β), 166.7 (CHO); Pro residue 176.2 (CO), 63.5 (α), 33.1 (β), 27.5 (γ), 51.6 (δ); **3** residue 174.7 (amide), 97.4 [$C(OH)_2$], 57.4 (α), 26.1 (β), 27.3 (γ), 43.7 (δ), 159.7 (guanidine). ^1H NMR data for **1** in D_2O: Phe residue 4.61 (dd, 4.9, 6.1; α), 2.77 (dd, 4.9, 13.6; β), 2.85 (dd, 6.1, 13.6; β′), 7.23 (2 H, m; 2, 6), 6.91 (2 H, t, 8.0; 3, 5), 7.25 (t, 8.0; 4); **4** residue 5.92 (td, 1.9, 15.6; α), 6.80 (td, 2.6, 15.6; β), 4.57 (m; γ), 2.50 (dd, 10.1, 14.2; δ), 2.98 (dd, 4.9, 14.2; δ′), 7.19 (2 H, d, 7.8; 2, 6), 6.66 (2 H, d, 7.8; 3, 5); Dpr residue 4.85 (m; α), 2.95 (m; β), 4.24 (ddd, 4.1, 10.1, 12.9; β′), 8.08 (s; CHO); Pro residue 4.52 (dd, 5.9, 5.9; α), 1.90 (m; β), 2.30 (m; β′), 1.86 (m; γ), 2.00 (m; γ′), 3.49 (m; δ), 3.76 (ddd, 6.7, 6.7, 10.2; δ′); **3** residue 4.00 (m; α), 1.55 (m; β), 1.93 (m, β′), 1.53 (m; γ), 1.66 (m; γ′), 3.17 (2 H, m; δδ′).

(17) For convenience in comparison with Arg, the methine carbon of **3** is designated as the α-carbon.

(18) Cyclotheonamide A decomposed during measurement of NMR spectra in DMSO-d_6: 10% of **1** was recovered by HPLC. Due to the instability in DMSO-d_6 solution, an HMBC spectrum of good quality was not obtained in this solvent.

(19) The following NOESY sequential cross peaks were observed: Phe-α, 4-NH; 4-α, Dpr-β-NH; Dpr-α, Pro-δ; Pro-α, 3-NH; 3-α, Phe-NH.

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(21) The following HMBC sequential cross peaks were observed: Phe-CO, 4-NH; 4-CO, Dpr-β; Dpr-CO, Pro-α; Pro-CO, 3-NH; 3-amide, Phe-α.

(22) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813. Selected ROESY sequential cross peaks: Phe-α, 4-NH; 4-α, Dpr-β-NH; Dpr-α, Pro-δ; Pro-α, 3-NH; 3-α, Phe-NH.

(23) Since the Dpr residue is epimerized during standard acidic hydrolysis as shown in our study on theonellamide F,[5] milder conditions (2 N HCl, 108 °C, 2 h) were used for the stereochemical analysis.

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Chelatoselective Fluorescence Perturbation in Anthrylazamacrocycle Conjugate Probes. Electrophilic Aromatic Cadmiation[1]

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Fluorescence methods offer many potential advantages in ion quantitation: sensitivity, ease of automation, and straightforward application to fiber optics based remote sensing techniques. While fluorimetric methods for the determination of some metal ions in aqueous solution exist using intrinsic probes,[3] selective methods for the determination of Zn(II) and Cd(II) do not. We have recently reported that anthrylazamacrocycle conjugate probes yield large (20–190-fold) changes in fluorescence upon metal ion complexation in aqueous solution;[4] the very large association constants between several transition metals (e.g., Pb(II), Cu(II), Zn(II), Cd(II), Hg(II)) and azamacrocycles[5] make the sequestration (and therefore quantitation) of small amounts of such ions possible. Only Zn(II) and Cd(II) bind anthrylazamacrocycles with net chelation-enhanced fluorescence (CHEF); however, assigning an enhancement to one metal or the other has not been possible heretofore. We now report that the complexation of Cd(II) and (anthrylmethyl)pentacyclen (**3**) *uniquely* demonstrates a perturbation of the fluorophore emission spectrum; the resulting ion discrimination can be utilized directly for simultaneous Zn(II)/Cd(II) analysis.

Normalized emission spectra for the complexes of Zn(II) and Cd(II) perchlorates with four homologous anthrylazamacrocycles[4] are shown in Figure 1. In each case an anthracenic fluorescence spectrum is observed. However, the Cd(II)–**3** complex displays an additional broad, red-shifted band yielding the composite spectrum with λ_{max} 446 nm. A typical anthracenic emission is observed for Zn(II) and Cd(II) complexes of (9′-anthryl-

(1) Presented at the 1989 International Chemical Congress of Pacific Basin Societies in Honolulu, HI, Dec 1989.

(2) Recipient of Ohio State University and Amoco graduate fellowships.

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