Three New Onnamide Congeners from the Okinawan Marine Sponge Theonella Sp.

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THREE NEW ONNAMIDE CONGENERS FROM THE OKINAWAN MARINE SPONGE *THEONELLA* SP.

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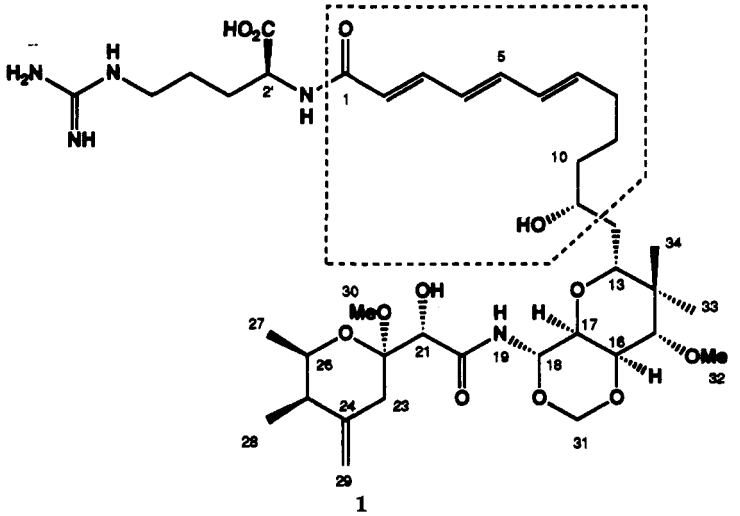
ABSTRACT.—Three new cytotoxic alkaloids, 6,7-dihydro-11-oxo-onnamide A [**2**], 11-oxo-onnamide A [**3**], and 4*Z*-onnamide A [**4**], have been isolated from an Okinawan marine sponge of the genus *Theonella* and their structures elucidated on the basis of spectroscopic data.

Sponges of the genus *Theonella* have produced a variety of bioactive secondary metabolites with unique chemical structures such as cyclic peptides (1–3) or macrocyclic bislactones (4–6). We have also reported the isolation and structural elucidation of the cyclic peptides konbamide (7) and keramamides A–D (8,9) and F (10) from the genus *Theonella*. Onnamide A [**1**], a potent antiviral compound, was first isolated from a sponge of the genus *Theonella* (11). During our investigations on bioactive substances from marine organisms (12–14), we have further examined the same *Theonella* sponge from which keramamides B–D were isolated and have obtained three new onnamide congeners, 6,7-dihydro-11-oxo-onnamide A [**2**], 11-oxo-onnamide A [**3**], and 4*Z*-onnamide A [**4**], together with onnamide A [**1**] and dihydro-onnamide A [**5**] (15). In this paper we describe the isolation and structure elucidation of **2–4**.

The sponge *Theonella* sp., collected off the Kerama Islands, Okinawa, was extracted with toluene-MeOH (1:3), and the extract was partitioned between toluene and H_2O. The $CHCl_3$-soluble material of the aqueous phase was subjected to flash chromatography on a Si gel column with $CHCl_3$-MeOH (1:1), followed by chromatography on an ODS column, preparative Si gel tlc, and reversed-phase hplc on an ODS column to afford 6,7-dihydro-11-oxo-onnamide A [**2**] (5.6×10^{-5}% wet wt of the sponge), 11-oxo-onnamide A [**3**] (3.0×10^{-5}%), and 4*Z*-onnamide A [**4**] (7.9×10^{-5}%), together with onnamide A [**1**], 8.8×10^{-4}%)



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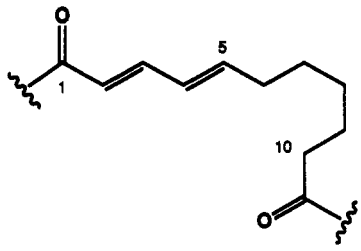

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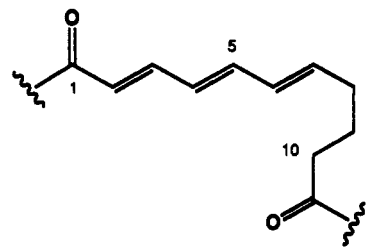

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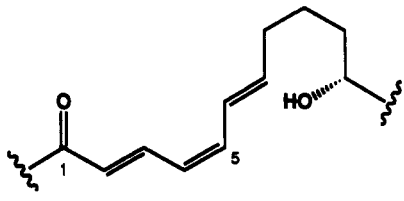

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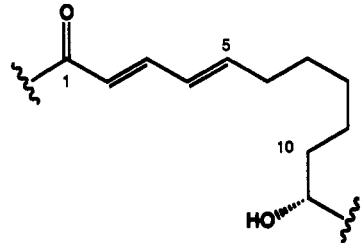

5

and 6,7-dihydro-onnamide A [**5**] (1.3×10^{-4}%).

Compound **2** was shown to have molecular formula $C_{39}H_{63}N_5O_{12}$ by the hrfabms (*m/z* 794.4565, $[M+H]^+$, Δ +1.3 mmu). The ir absorptions at 3400 and 1650 cm^{-1} indicated the presence of hydroxy and/or amino group(s) and amide group(s), respectively. The uv spectrum (λ max 263 nm) was indicative of the presence of a dienone moiety. The ^{1}H-nmr (Table 1) spectrum revealed that **2** possessed an exo-methylene (δ_H 4.63, s and 4.79, s), a conjugated diene (δ_H 6.01, 6.08, 6.20, and 7.10), two methoxy groups (δ_H 3.24, s and 3.55, s), and four methyl groups (δ_H 0.86, 0.95, 1.03, and 1.18). In the ^{13}C-nmr spectrum of **2** (Table 2), a quaternary carbon signal at δ 158.6 was assigned as a guanidine carbon of arginine. These spectral data resembled those of onnamide A [**1**] except for ^{1}H and ^{13}C resonances in the olefinic region. The blue shift of the uv spectra (λ max 298 nm in **1** to 263 nm in **2**) suggested differences in a chromophore (C-1–C-7) between **1** and **2**. The ^{1}H-^{1}H COSY spectrum revealed the presence of a conjugated diene (C-2–C-5) which had all *E* geometry judging from the ^{1}H-^{1}H coupling constants ($J_{2,3}$=15.3 Hz and $J_{4,5}$=14.7 Hz). The carbon chemical shift (δ_C 71.0 in **1** to δ_C 210.8 in **2**) of C-11 indicated that the hydroxyl group at C-11 in **1** was oxidized to be a ketone group. As a result, compound **2** was elucidated to be 6,7-dihydro-11-oxo-onnamide A.

The molecular formula of compound **3** was determined to be $C_{39}H_{61}N_5O_{12}$, by the hrfabms (*m/z* 792.4431 $[M+H]^+$, Δ +3.6 mmu) which was less than that of **1** by 2 daltons. The ^{1}H-nmr (Table 1) spectrum was very similar to that of compound **1**. The presence of a ketone group (C-11) was revealed by comparison of the chemical shifts of H-12 (δ_H 2.49) and H-13 (δ_H 3.95) in **3** with those in **1** and by lack of an H-11 signal for **3**. Thus compound **3** was assigned as 11-oxo-onnamide A.

Compound **4** was shown to have the molecular formula $C_{39}H_{63}N_5O_{12}$ by the hrfabms (*m/z* 794.4593 $[M+H]^+$, Δ +4.1 mmu) which was the same as that of onnamide A [**1**]. Six olefinic protons were observed at δ_H 6.06 (H-2), 7.66 (H-3), 5.99 (H-4), 6.27 (H-5), 6.70 (H-6), and 5.96 (H-7), which were assignable to a conjugated triene group on the basis of

TABLE 1. ^{1}H-nmr Data of Compounds **2–4** in CD_3OD.[a]

Proton	Compound		
	2	**3**	**4**
H-1	—	—	—
H-2	6.01 (d, 15.3)	6.08 (d, 14.8)	6.06 (d, 14.4)
H-3	7.10 (dd, 15.3, 10.7)	7.14 (dd, 14.8, 11.3)	7.66 (dd, 14.4, 11.9)
H-4	6.20 (dd, 14.7, 10.7)	6.28 (dd, 14.9, 11.3)	5.99 (dd, 11.9, 10.7)
H-5	6.08 (dt, 14.7, 6.4)	6.52 (dt, 14.9, 10.4)	6.27 (dd, 11.7, 10.7)
H-6	2.18 (m)	6.18 (dd, 15.1, 10.4)	6.70 (dd, 14.5, 11.7)
H-7	1.43 (m)	5.88 (dt, 15.1, 7.0)	5.96 (dt, 14.5, 7.3)
H-8	1.52 (m)	2.18 (dd, 14.6, 7.0)	2.18 (m)
H-9	1.62 (m)	1.62 (m)	1.45 (m)
		1.60 (m)	1.60 (m)
H-10	2.36 (m)	2.44 (m)	1.53 (m)
H-11	—	—	3.64 (m)
H-12	2.48 (m)	2.49 (m)	1.30 (m)
			1.45 (m)
H-13	3.96 (dd, 8.5, 3.2)	3.95 (dd, 8.8, 3.4)	3.48 (dd, 8.3, 3.9)
H-14	—	—	—
H-15	3.63 (m)	3.60 (m)	3.67 (m)
H-16	4.14 (dd, 9.1, 6.1)	4.14 (dd, 9.3, 5.9)	4.16 (dd, 9.8, 6.3)
H-17	3.88 (m)	3.89 (m)	3.98 (dd, 9.3, 6.3)
H-18	5.74 (d, 8.3)	5.74 (d, 8.8)	5.80 (d, 9.3)
H-19	—	—	—
H-20	—	—	—
H-21	4.29 (s)	4.28 (s)	4.24 (s)
H-22	—	—	—
H-23	2.28 (d, 14.6)	2.28 (d, 14.1)	2.31 (d, 14.7)
	2.38 (d, 14.6)	2.38 (d, 14.1)	2.39 (d, 14.7)
H-24	—	—	—
H-25	2.18 (m)	2.20 (dd, 6.8, 2.4)	2.18 (m)
H-26	3.88 (m)	3.87 (m)	3.85 (dd, 6.4, 2.4)
H-27	1.18 (d, 6.4)	1.19 (d, 6.8)	1.16 (d, 6.4)
H-28	0.95 (d, 6.8)	0.95 (d, 6.8)	0.95 (d, 7.3)
H-29	4.63 (s)	4.63 (s)	4.63 (s)
	4.79 (s)	4.79 (s)	4.79 (s)
H-30	3.24 (s)	3.24 (s)	3.22 (s)
H-31	4.87 (d, 6.8)	4.87 (d, 7.3)	4.87 (d, 6.8)
	5.16 (d, 6.8)	5.16 (d, 7.3)	5.21 (d, 6.8)
H-32	3.55 (s)	3.54 (s)	3.56 (s)
H-33	0.86 (s)	0.86 (s)	0.86 (s)
H-34	1.03 (s)	1.02 (s)	1.00 (s)
H-1′	—	—	—
H-2′	4.37 (m)	4.36 (dd, 7.5, 5.2)	4.39 (dd, 7.3, 5.4)
H-3′	1.73 (m)	1.74 (m)	1.75 (m)
	1.89 (m)	1.90 (m)	1.90 (m)
H-4′	1.62 (m)	1.62 (m)	1.62 (m)
H-5′	3.21 (m)	3.21 (m)	3.24 (m)
H-6′	—	—	—
H-7′	—	—	—

[a]δ in ppm, multiplicity, J in Hz.

the ^{1}H-^{1}H COSY spectrum. The coupling constant (J=10.7 Hz) between H-4 and H-5 revealed a Z configuration of the C-4 double bond, while an E configuration was indicated for the C-2 double bond ($J_{2,3}$=14.4 Hz) as well as the C-6

TABLE 2. ^{13}C-nmr Data of Compounds **2–4** in CD_3OD.[a]

Carbon	Compound		
	2	**3**	**4**
C-1	168.4 (s)	168.2 (s)	168.2 (s)
C-2	143.9 (d)[b]	124.4 (d)	140.9 (d)[b]
C-3	142.2 (d)[b]	142.0 (d)	137.7 (d)[b]
C-4	130.0 (d)[b]	129.7 (d)	136.7 (d)[b]
C-5	123.3 (d)[b]	141.0 (d)	126.7 (d)[b]
C-6	30.8 (t)	131.9 (d)	125.8 (d)[b]
C-7	30.8 (t)	139.6 (d)	125.1 (d)[b]
C-8	34.4 (t)	33.2 (t)	33.9 (t)
C-9	26.0 (t)	26.1 (t)	26.0 (t)
C-10	43.8 (t)	43.1 (t)	36.9 (t)
C-11	210.8 (s)	210.5 (s)	71.1 (d)
C-12	43.5 (t)	43.1 (t)	37.4 (t)
C-13	70.5 (d)	76.4 (d)	78.7 (d)
C-14	42.1 (d)	43.0 (d)	42.1 (d)
C-15	81.1 (d)	81.0 (d)	80.6 (d)
C-16	76.4 (d)	75.3 (d)	75.6 (d)
C-17	70.9 (d)	70.9 (d)	70.8 (d)
C-18	75.3 (d)	75.0 (d)	74.9 (d)
C-19	—	—	—
C-20	174.1 (s)	174.1 (s)	174.4 (s)
C-21	73.4 (d)	73.5 (d)	74.0 (d)
C-22	101.4 (s)	101.4 (s)	101.3 (s)
C-23	34.9 (t)	34.5 (t)	34.8 (t)
C-24	148.2 (s)	148.2 (s)	148.2 (s)
C-25	43.0 (d)	43.6 (d)	43.0 (d)
C-26	70.9 (d)	70.9 (d)	70.8 (d)
C-27	18.1 (q)	18.2 (q)	18.1 (q)
C-28	12.6 (q)	12.6 (q)	12.4 (q)
C-29	110.2 (t)	110.2 (t)	110.0 (t)
C-30	48.8 (q)	48.8 (q)	48.8 (q)
C-31	87.4 (t)	87.5 (t)	87.6 (t)
C-32	61.7 (q)	61.7 (q)	61.9 (q)
C-33	14.4 (q)	15.4 (q)	14.2 (q)
C-34	24.4 (q)	24.0 (q)	23.6 (q)
C-1′	178.6 (s)	178.4 (s)	178.4 (s)
C-2′	55.5 (d)	55.4 (d)	55.5 (d)
C-3′	31.4 (t)	31.4 (t)	31.4 (t)
C-4′	26.1 (t)	26.1 (t)	26.1 (t)
C-5′	42.1 (t)	42.1 (t)	42.1 (t)
C-6′	—	—	—
C-7′	158.6 (s)	158.6 (s)	158.6 (s)

[a]δ in ppm.
[b]Assignments may be interchanged.

double bond ($J_{6,7}$=14.5 Hz). Except for this olefinic moiety, the ^{1}H and ^{13}C nmr (Table 1 and 2) of **4** resembled those of **1**. Hence, compound **4** was concluded to be the 4*Z* isomer of onnamide A [**1**].

The stereochemistry of compound **4** was established by the interpretation of the ^{1}H-^{1}H coupling constants and the NOESY spectrum. The cis configuration between the tetrahydropyran ring (C-13–C-17) and the dioxane ring was confirmed by the NOESY correlation between H-16 and H-17. The tetrahydropyran ring was in a chair con-

formation with an equatorial C-13 substituent, judging from the NOESY cross peaks of H-13/H-15, H-13/H-18, H-15/H-31, and H-16/H_3-33. The β configuration of H-18 was assigned by the ^1H-^1H coupling constants between H-18 and H-17 (9.3 Hz) and the NOESY correlation between H-18 and H-13.

The ^1H-^1H coupling constants and the ^{13}C nmr data of the C-13–C-34 moiety in compounds **2–4** were similar to those of onnamide A [**1**]. Thus the stereochemistry of compounds **2–4** was identical with that of **1**.

Compounds **1–5** exhibited cytotoxicity against murine lymphoma L1210 cells in vitro with IC_{50} values of 0.002, 0.016, 0.0092, 0.0015, and 0.0046 μg/ml, respectively, and human epidermoid carcinoma KB cells in vitro with IC_{50} values of 0.0036, 0.023, 0.013, 0.0029, and 0.005 μg/ml, respectively. The cytotoxicity of compound **4** was essentially the same as that of onnamide A [**1**], and the activities of compounds **2**, **3**, and **5** were all less than that of **1**.

EXPERIMENTAL

GENERAL PROCEDURES.—Optical rotations were measured on a DIP-370 polarimeter. Uv and ir spectra were taken on a Shimadzu UV-220 and a JASCO IR Report-100 spectrometer, respectively. ^1H- and ^{13}C-nmr spectra were recorded on a JEOL EX-400 spectrometer in CD_3OD. The 3.30 ppm resonance of residual CD_2HOD and 49.0 ppm resonance of CD_3OD were used as internal references for ^1H and ^{13}C chemical shifts, respectively. Fabms spectra were obtained on a JEOL JMS-HX110 spectrometer with glycerol as a matrix. Wako C-300 Si gel (Wako Pure Chemical) was used for glass cc, and tlc was carried out on Merck Si gel GF_{254}.

SPONGE MATERIAL.—The sponge *Theonella* sp. (suborder Tetracladina, Family Theonellidae) was collected by scuba off Kerama Island, Okinawa and kept frozen until used. The sponge had a thin medium brown layer at the surface firmly adhered to the underlying mesohyl which is light yellow when preserved. The mesohyl is very dense and compact. The skeleton consists of tracts aligned at right angles to the surface containing 2–6 spicules. Principal megascleres are strongyles or strongyloxeas measuring 312–456×6–10 μm. Small spiny microstrongyles are 9 μm long, phyllotriaenes 170 μm across, desmas 190 μm across. The voucher specimen (SS-246) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

COLLECTION, EXTRACTION, AND ISOLATION.—The toluene-MeOH (1:3) extract (1 liter×2) of the sponge (4.0 kg wet wt) was suspended in 1M NaCl (1 liter) and was extracted with toluene (600 ml×2). The aqueous layer was subsequently extracted with $CHCl_3$ (800 ml×2). The $CHCl_3$-soluble fraction was evaporated under reduced pressure to give a crude residue (2.1 g), which was subjected to a Si gel column (4.5×36 cm) with gradient elution of MeOH (2–50%) in $CHCl_3$. The fraction eluted with 50% MeOH in $CHCl_3$ was then separated by reversed-phase cc on ODS (YMC-GEL I-40/64, 60 Å, Yamamura Chemical, 2.8×24 cm) with MeCN-H_2O (30:70). The fraction eluting from 180 to 300 ml was further separated by a preparative Si gel tlc with $CHCl_3$-MeOH-H_2O (65:25:4) and subsequently subjected to reversed-phase cc on YMC-ODS (1.3×15 cm) with MeOH-H_2O (65:35) to give a crude onnamide fraction (15–75 ml). Moreover, the sponge (4 kg wet wt) was also extracted and separated by the same method as described above to give a crude onnamide fraction, which amounted to 160.9 mg in all. The fraction was purified by reversed-phase hplc [YMC-Pack AM-323 ODS, Yamamura Chemical, 1.0×25 cm; flow rate, 3.0 ml/min; uv detection at 254 nm; eluent MeOH-H_2O (62:38)] to afford onnamide A [**1**] (70.7 mg, Rt 25.5 min), fraction A (19.8 mg, Rt 29.0 min), 6,7-dihydro-11-oxo-onnamide A [**2**] (4.5 mg, Rt 41.0 min), 4*Z*-onnamide A [**4**] (6.3 mg, Rt 31.5 min), and dihydroonnamide A [**5**] (10.7 mg, Rt 34.0 min). The fraction A was purified by the same reversed-phase hplc described above [eluent MeCN-H_2O (35:65)] to give 11-oxo-onnamide A [**3**] (2.4 mg, Rt 10.3 min).

6,7-Dihydro-11-oxo-onnamide A [**2**].—Colorless soild: $[\alpha]^{24}D$ +39° (*c*=0.42, MeOH); uv (MeOH) λ max 262 nm (ε 22700); ir (KBr) ν max 3400, 2920, 1650, 1380, 1100, 1020 cm−1; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* $[M+H]^+$ 794; hrfabms *m/z* $[M+H]^+$ 794.4565 (calcd for $C_{39}H_{63}N_5O_{12}$, 794.4552).

11-Oxo-onnamide A [**3**].—Colorless solid: $[\alpha]^{23}D$ +90° (*c*=0.24, MeOH); uv (MeOH) λ max 298 nm (ε 39700); ir (KBr) ν max 3400, 2920, 1650, 1380, 1100, 1020 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* $[M+H]^+$ 792; hrfabms *m/z* $[M+H]^+$ 792.4431 (calcd for $C_{39}H_{61}N_5O_{12}$, 792.4395).

4Z-Onnamide A [**4**].—Colorless solid: $[\alpha]^{23}D$ +81° (*c*=0.59, MeOH); uv (MeOH) λ max 300 nm (ε 25800); ir (KBr) ν max 3400, 2920, 1650, 1380, 1090, 1020 cm^{-1}; ^1H nmr see Table 1; ^{13}C

nmr see Table 2; fabms *m/z* $[M+H]^+$ 794; hrfabms *m/z* $[M+H]^+$ 794.4593 (calcd for $C_{39}H_{63}N_5O_{12}$, 794.4552).

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