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Eight New Cytotoxic Metabolites Closely Related to Onnamide A from Two Marine Sponges of the Genus *Theonella*[1]

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Abstract: New cytotoxic compounds **1-7** were isolated along with onnamide A (**9**) from a marine sponge *Theonella* sp. collected at Hachijo Island, whereas another *Theonella* sponge yielded **8**. The structures of these compounds were determined by interpretation of the NMR spectral data as well as by comparison of spectral data with those of **9**. Six compounds (**1-6**) were highly cytotoxic against the P388 cell line.

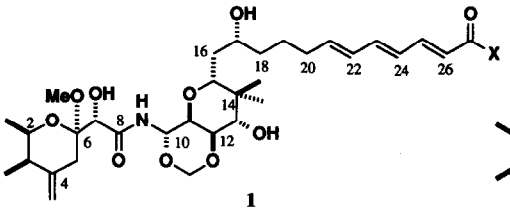
Marine sponges of the genus *Theonella* have proved to contain a diverse array of secondary metabolites possessing various biological activities; e. g. swinholides,[2] bistheonellides,[3] theonellamide F,[4] and theonellapeptolides.[5] We have already reported cyclotheonamides,[6] orbiculamide A,[7] aurantosides,[8] and theopederins[9] from a *Theonella* sponge yellowish inside collected off Hachijo Island. Further examination of the water soluble portion of the EtOH extract led to the isolation of eight cytotoxic metabolites which include onnamide A[10] and closely related compounds.

The EtOH extract of the sponge was partitioned between water and ether, and the aqueous phase was extracted with *n*-BuOH. The MeOH soluble portion of the *n*-BuOH extract was fractionated by Sephadex LH-20 and ODS chromatographies to afford **1-7** along with onnamide A (**9**). Similarly, another species of a *Theonella* sponge, which was also collected off Hachijo Island, gave rise to pseudoonnamide A (**8**),[11] also related to onnamide A. Compounds, **1-6** and **9** were highly cytotoxic against the P388 cell line with IC_{50} values of 0.15, 0.04, 0.13, 0.10, 0.07, 0.02, and 0.01 μg/mL, respectively.

13-Des-*O*-methyl-onnamide A (**1**), the most polar of the 9 metabolites, had a molecular formula of $C_{38}H_{61}N_5O_{12}$, which was one CH_2 unit smaller than onnamide A. The ^1H and ^{13}C NMR spectra (Tables I and II) were almost superimposable on those of onnamide A, except for signals assignable to the C13*O*-methyl group, which were missing in **1**. In addition, the C13 signal in **1** was shifted upfield (δ 69.8 vs. δ 80.6 in **9**), while the H13 signal was shifted downfield (δ 4.01 vs. δ 3.62 in **9**). This indicated that the C13*O*-methyl group in onnamide A was replaced by a hydrogen atom in **1**. Interestingly, ^{13}C NMR signals for 14-Me and C11 and ^1H NMR signal for H13, which were broad in onnamide A and related compounds described in this paper, were sharp in **1**.[12]

Dihydroonnamide A (**2**) displayed an HPLC peak with the longest retention time among the nine metabolites. A molecular formula of $C_{39}H_{65}N_5O_{12}$ was deduced from high resolution FAB mass spectroscopy, indicating that one of double bonds in onnamide A had been reduced. This deduction was also supported by the ^1H and ^{13}C NMR spectra and by the UV spectrum (λmax 260 nm), which revealed the presence of a conjugated diene system instead of a conjugated triene in **9**. Interpretation of the COSY, HMQC,[13] and HMBC[13] spectra showed that O1 to C17, C23 to C27, and arginyl amide portions of **2** were identical with those of onnamide A. The remaining five methylenes could be placed between C17 and C23. Though connectivities $H17/H_218$ and H_221/H_222 were readily secured by COSY spectrum, the assignment of other methylene signals was hampered due to a severe overlapping of the signals, which was overcome by a relayed-COSY experiment,[14] which showed correlation peaks between H17 and H_219 and between H_222 and H_220. Therefore structure **2** was secured for dihydroonnamide A.

Onnamide B (**3**) was smaller than onnamide A by a C_2H_2 unit. The UV spectrum (λmax 260 nm) indicated the presence of an α, β, γ, δ-unsaturated carbonyl chromophore as in **2**. Interpretation of the COSY and HMQC spectra revealed that onnamide B was a derivative of onnamide A with one less double bond.



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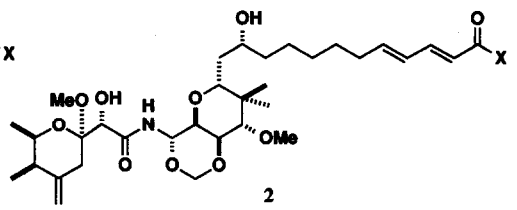

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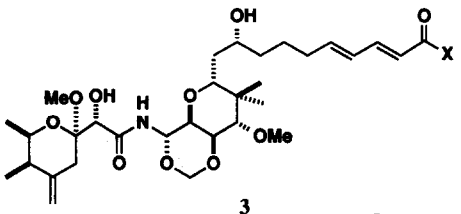

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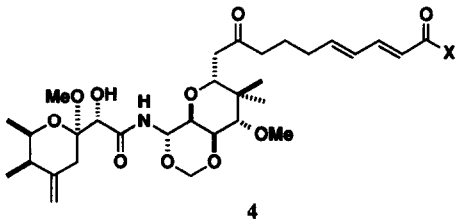

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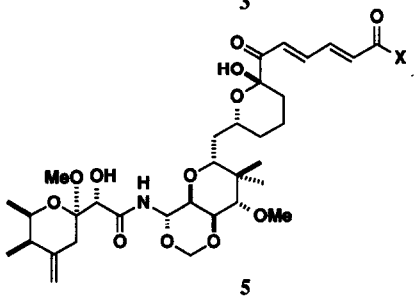

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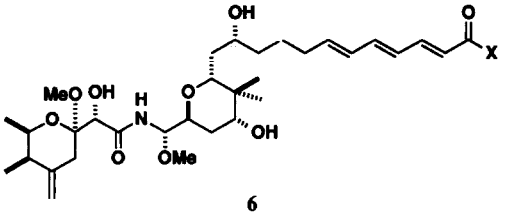

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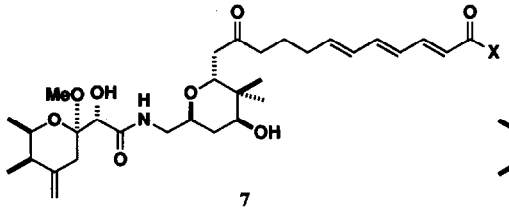

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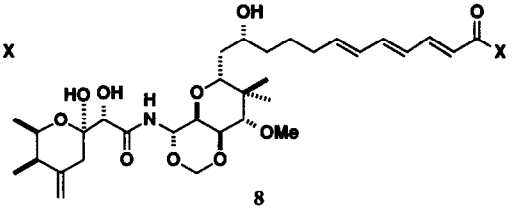

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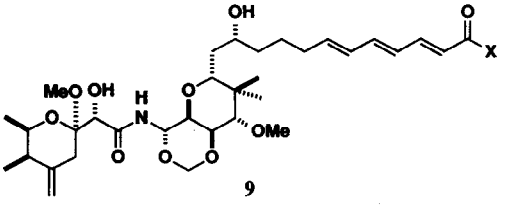

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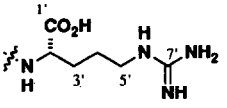
X= 

High resolution FAB mass spectrum of 17-oxoonnamide B (**4**) revealed a molecular formula of $C_{37}H_{59}N_5O_{12}$, two hydrogens less than **3**. The NMR data readily showed that a secondary alcohol in **3** was replaced by a ketone (δ 210.4). Considerable downfield shifts were observed for $H_2$16 (δ 2.46 and 2.53 vs. 1.53 (2H) in **3**) and $H_2$18 (δ 2.40 and 2.48 vs. 1.29 and 1.49 in **3**), which were assigned by the COSY spectrum, while a signal for the H17 carbinol methine proton was missing. The ketone carbon was correlated with H15, $H_2$16, $H_2$18, and $H_2$19 in the HMBC spectrum, allowing assignment of **4** as 17-oxoonnamide B.

Onnamide C (**5**) had a molecular formula of $C_{39}H_{61}N_5O_{12}$ indicating 12 degrees of unsaturation. The ^1H and ^{13}C NMR and COSY spectra showed that the O1 to C16 portion and the arginyl amide in **5** were identical with those of onnamide A. In sharp contrast with the other metabolites, the C23-C26 conjugated diene unit [δ 6.54 (d, *J*=15.0 Hz), 7.26 (dd, 11.4, 15.0), 7.38 (dd, 11.4, 15.3), 7.00 (d, 15.3)] was without vicinal protons. The ^{13}C NMR spectrum contained signals for a ketone (δ 198.2) and an acetal or hemiacetal (δ 97.9). Interpretation of the COSY spectrum revealed the ^1H coupling network from $H_2$16 to $H_2$20, in which H17 (δ 4.04) was shifted downfield and the methylene protons on C18 were strongly non-equivalent (δ 1.15 and 1.74). In compounds **1-4**, H17 appeared between δ 3.62-3.66 and the chemical shift difference between the C18 methylene protons was less than 0.2 ppm. The HMBC spectrum with an evolution time of 60 msec revealed cross peaks between C22 (δ 198.2) and both H23 and H24, thereby allowing us to place a ketone on C22. Though the signal at δ 97.9 showed no cross peaks in the HMBC spectrum with an evolution time of 60 msec, this carbon signal gave a cross peak with one of the C20 methylene protons (δ 1.60) in the HMBC spectrum with an evolution time of 100 msec. Therefore, C20 had to be adjacent to the hemiacetal/acetal carbon, which in turn was linked to C22. In order to account for the degree of unsaturation, C17 and C21 were connected by an oxygen to form a tetrahydropyran ring, which was consistent with the non-equivalence of the C18 methylene protons and the NOESY data described below.

Table I. Selected ^1H NMR Data for **1**, **4**, **5**, **6**, and **7**

#H	1	4	5	6	7
H10	5.81 (9.8)	5.74 (8.5)	5.78 (9.2)	5.36 (7.6)	3.31 m
H10					3.52 (4.1, 13.7)
10-OCHa	5.11 (7.0)	5.16 (6.9)	5.20 (7.0)	3.37	
10-OCHe	4.73 (7.0)	4.79 (6.9)	4.80 (7.0)		
H11	4.03 (6.6, 9.8)	3.87 (6.0, 8.5)	3.95 (7.5, 9.2)	3.92 (3.1, 6.9, 7.6)	3.83 m
H12a	4.08 (6.6, 10.5)	4.14 (6.0, 9.1)	4.15 (7.5, 9.8)	1.74 (6.9, 10.5,13.7)	1.47 (8.5, 8.5,13.5)
H12e				1.98 (3.1, 4.6, 13.7)	1.86 m
H13	4.01 (10.5)	3.59 br (9.1)	3.63 brd (9.8)	3.59 (4.6, 10.5)	3.63 (4.2, 8.5)
13-OCH3		3.55	3.55		
14CH3e	0.98	1.02	1.00	0.92	0.90
14CH3a	0.88	0.86	0.85	0.85	0.98
H15	3.45 (4.8, 8.1)	3.95 (3.0, 9.3)	3.45 (1.8, 10.0)	3.44 (2.0, 10.3)	4.17 (3.5, 10.4)
H16	1.53 m	2.47 (3.0, 16.0)	1.54 (1.8, 9.0, 14.2)	1.56 m	2.57 (3.5, 14.5)
H16	1.53 m	2.53 (9.3, 16.0)	1.60 m	1.63 m	2.77 (10.4, 14.5)
H17	3.65 m		4.04 (2.1, 3.5, 9.2,11.7)	3.66 m	
H18	1.30 m	2.42 m	1.15 m	1.37 m	2.49 (7.3, 7.3,17.4)
H18	1.48 m	2.47 m	1.75 m	1.46 m	2.57 m
H19	1.42 m	1.64 m	1.71 m	1.46 m	1.70 quint (7.3)
H19	1.55 m	1.64 m	1.88 m	1.56 m	1.70 quint (7.3)
H20	2.14 m	2.14 brq (7.5)	1.60 m	2.19 brq (6.9)	2.16 q (7.2)
H20	2.20 m	2.14 brq (7.5)	1.74 m	2.19 brq (6.9)	2.16 q (7.2)
H21	5.94 (7 0, 7.0, 14.8)	6.04 (7.0, 7.0, 15.0)		5.93 (7.0, 7.0, 14.7)	5.89 (7.0, 7.0,15.1)
H22	6.21 (10.7, 14.8)	6.21 (10.7, 15.0)		6.19 (14.7, 10.7)	6.19 (10.6, 15.1)
H23	6.52 (10.7, 14.8)	7.09 (10.8, 15.2)	7.00 (15.3)	6.52 (10.7, 14.8)	6.53 (10.6, 14.8)
H24	6.26 (11.0, 15.0	6.02 (15.2)	7.38 (11.4, 15.3)	6.26 (11.2, 14.8)	6.28 (11.3, 14.8)
H25	7.14 (11.0, 15.0)		7.26 (11.4, 15.0)	7.14 (11.2, 15.2)	7.14 (11.3, 15.0)
H26	6.06 (15.0)		6.54 (15.0)	6.06 (15.2)	6.08 (15.0)

Onnamide D (**6**) possessed a molecular formula of $C_{38}H_{63}N_5O_{11}$, as determined by high resolution FAB mass spectroscopy. This compound had one degree of unsaturation less than onnamide A. As the sp^2 carbon region of the ^{13}C NMR spectrum of **6** was almost superimposable on that of **9**, onnamide D appeared to have one less ring than onnamide A. Analysis of the 2D NMR data

disclosed that O1 to C8, C15 to C27, and the arginyl amide portions were those of **9**. Onnamide D showed no signals for the dioxymethylene group in the ^{1}H and ^{13}C NMR spectra, and signals for an *O*-methyl group (δ_H 3.37; δ_C 56.5) were apparently different from those assigned to the *O*-methyl group on C13 (δ_H 3.55; δ_C 61.9) in onnamide A. Interpretation of the COSY spectrum revealed that H10 was shifted upfield by 0.44 ppm and C12 was a methylene (δ_H 1.74, 1.98; δ_C 30.3). The HMBC spectrum indicated that the *O*-methyl group was attached to C10; the ^{13}C chemical shift for C13 (δ 71.8) suggested that the methoxy group was replaced by a free hydroxyl group on this carbon. An HMBC cross peak between H11 and C15 secured the presence of a tetrahydropyranyl ring. Interestingly, the O1 to C15 portion of onnamide D has the same gross structure as pederin, the toxic principle of blister beetles [10](*vide infra*).

Table II. ^{13}C NMR Data of **1-8** in CD_3OD

#C	1	2	3	4	5	6[a]	7[a]	8[a]
2	70.9	70.9	70.8	70.9	70.9	70.3	70.5	69.8
2-Me	18.1	18.1	18.2	18.2	18.1	18.0	18.0	18.1
3	43.0	43.0	43.0	43.0	43.0	42.6	42.8	43.1
3-Me	12.3	12.4	12.4	12.6	12.3	12.3	12.1	12.0
4	148.2	148.2	148.1	148.2	148.2	148.0	147.7	148.4
4=CH2	110.1	110.1	110.1	110.3	110.1	109.8	109.9	109.8
5	34.7	34.8	34.7	34.5	34.7	34.7	33.7	36.1
6	101.3	101.3	101.3	101.4	101.4	100.9	101.0	93.9
6-OMe	48.8	48.7	48.8	48.	48.8	48.8	48.2	
7	74.2	74.1	73.9	73.4	73.9	74.2	72.4	76.6
8	174.4	174.3	174.4	174.1	174.3	174.4	173.0	175.3
10	74.6	74.9	74.9	75.3	74.8	80.6	43.0	74.4
10-OCH2	87.3	87.7	87.6	87.4	87.5	56.5		87.2
11	71.7	70.9	70.8	70.2	70.9	73.1	70.4	71.0
12	75.0	75.5	75.5	75.0	75.6	30.3	33.5	75.4
13	69.8	80.6	80.6	81.0	80.8	71.8	71.9	80.0
13-OMe		61.9	61.9	61.7	61.9			61.6
14	42.0	42.1	42.2	41.4	42.1	39.3	42.8	42.5
14-Me	13.2	14.4	14.3	15.4	14.4	13.9	20.8	13.8
14-Me	23.3	23.7	23.6	24.0	23.7	23.5	22.2	23.2
15	78.8	79.0	78.7	76.4	76.3	81.0	75.3	78.5
16	37.6	37.4	37.2	43.5	36.6	36.8	42.1	37.0
17	71.2	71.4	71.0	210.4	69.9	72.4	211.3	71.0
18	36.7	37.4	36.8	43.0	30.7	37.2	42.9	36.8
19	26.2	26.4	25.9	23.7	19.5	26.0	23.8	25.7
20	33.9	30.5	33.9	33.2	31.2	33.6	33.0	33.7
21	140.6	30.2	144.1	143.2	97.9	140.0	139.2	140.3
22	131.4	34.0	129.9	130.4	198.2	131.2	131.9	132.3
23	141.3	144.0	142.2	142.0	130.5	140.9	140.6	141.3
24	129.4	129.9	123.4	123.7	142.2	129.1	129.5	128.9
25	142.1	142.2	168.3	168.3	138.8	142.0	141.7	142.2
26	124.1	123.4			133.9	123.8	124.1	123.3
27	168.3	168.3			166.8	168.0	168.1	168.3
1'	178.3	178.6	178.7	178.4	178.2	177.9	177.9	175.9
2'	55.2	55.4	55.5	55.4	55.7	55.1	55.1	53.6
3'	31.2	31.4	31.3	31.4	31.2	31.0	31.2	30.6
4'	26.2	26.4	26.2	26.1	26.2	25.8	25.8	26.0
5'	42.0	42.1	42.0	42.1	42.1	41.9	41.8	41.7
7'	158.6	158.6	158.6	158.6	158.6	158.3	158.3	158.3

[a]. Chemical shifts were determined by tracing the projection of HMQC and HMBC spectra.

Onnamide E (**7**), $C_{37}H_{59}N_5O_{12}$, contains a ketone (δ 211.3) and an *O*-methyl group, but lacked the dioxymethylene group of **5**. In onnamide E O1 to C8, C21 to C27, and arginyl amide moieties were identical with those of **9**, which was disclosed by COSY and HMQC data. Further analysis of the COSY spectrum indicated that C16 (δ_H 2.57, 2.77) and C18 methylenes (δ_H 2.49, 2.57) were adjacent to a ketone (δ 211.3), which was proved by HMBC cross-peaks, viz. C17/$H_2$16, C17/$H_2$18, and C17/$H_2$19. In the ^{1}H NMR spectrum of **7** measured in CD_3OD, a characteristic doublet signal for H10 was replaced by methylene protons (δ 3.31 and

3.52), both of which gave COSY cross peaks with H11 at δ 3.83. H11 was further correlated with $H_2$12 (δ 1.47, 1.86), which in turn was coupled to H13 (δ 3.63); therefore no oxygen atom could be on either C10 or C12. There was an HMBC cross peak between H15 and C11, which proved the presence of a tetrahydropyran.

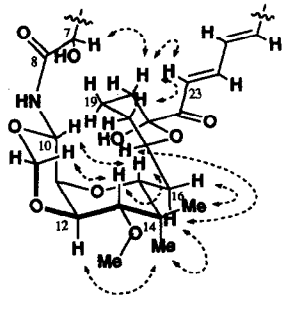
Pseudoonnamide A (**8**) had a molecular formula of $C_{38}H_{61}N_5O_{12}$, identical with **1**. Since the ^{1}H NMR spectrum showed only one *O*-methyl group, **8** had a structure in which one *O*-methyl group was missing from onnamide A. The major difference in the ^{1}H NMR spectra between **1** and **9** lay in the chemical shift for $H_2$5 and H7 and the absence of an *O*-methyl on C6: chemical shift differences between the C5 methylene protons were between 0.06 and 0.12 ppm in **1-7** and the difference in **8** was 0.63 ppm; the H7 signal appeared between 4.22 and 4.28 ppm in **1-7** and at 3.95 ppm in **8**. Those data indicated a 6-des-*O*-methylonnamide A structure for **8**, which was supported by extensive 2D NMR experiments including COSY, HMQC, and HMBC.

Stereochemistry. The ^{1}H-^{1}H coupling constants and the ^{13}C NMR data, which were almost superimposable on those of onnamide A, whose stereochemistry was established by synthesis,[15] indicated that the stereochemistry of the O1 to C17 portion of **1-4** was identical with that of **9**. Co-occurrence of onnamide A ($[\alpha]^{23}_D$, +64°, *c* 0.1, MeOH) in this sponge suggested that the Arg portion of **1-8** had the same *S* stereochemistry at the α-methine carbon.

The stereochemistry of onnamide C could be established by the interpretation of the NOESY and ROESY data (Scheme 1). Analysis of the coupling constants between H15 and $H_2$16 (H15/H16a *J*= 1.8 Hz; H15/H16b *J*= 10.0 Hz) and ROESY cross-peaks (14-CH_3a/H16a; 14-CH_3a/H16b; 14-CH_3e/H16b) revealed that the C14-C15 bond was antiperiplanar to the C16-C17 bond, whereas coupling constants between $H_2$16 and H15 (H16a/H17 *J*= 9.2 Hz; H16b/H17 *J*= 2.1 or 3.5 Hz) and ROESY cross-peaks between H15 and H18a and between H10 and H18e allowed correlation of the stereochemistry of C17 with that of C15. A ROESY cross-peak between the axial proton on C20 and H23 indicated that the hydroxyl group on C21 was axial. Interestingly enough, there were ROESY cross-peaks between H7 and $H_2$19/$H_2$20.

Coupling constants observed for the C10-C16 portion of onnamide D indicated that the tetrahydropyran ring was in chair conformation with axial C11 and equatorial C15 substituents, as observed in pederin by X-ray crystallography.[10c] NOESY and ROESY cross peaks expected from this conformation were observed. (Scheme 1) A ROESY cross peak between H7 and H17 allowed us to assign the stereochemistry at C10. The magnitude of coupling constants between H10 and H11 (9.2 Hz) supported this assignment.[16]

Scheme 1. Conformation of the central portion of **5-7** as shown by NOESY spectra



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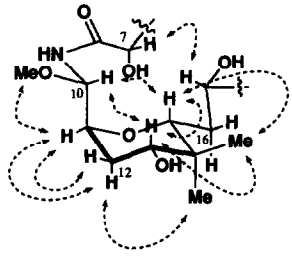

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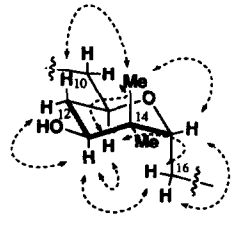

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In onnamide E both H11 and H13 were axial judging from coupling constants of 8.5 Hz between these protons and the axial proton on C12 and from a NOESY cross-peak between H11 and H13. On the other hand, both H11 and H13 gave NOESY cross-

peaks with one of the C16 methylene protons, indicating that the C15 substituent was axial. Therefore, onnamide E had a chair conformation flipped from that of onnamide D in the C11-C15 tetrahydropyran ring with equatorial C11 and axial C15 substituents.(Scheme 1) Interestingly, C13 stereochemistry of onnamide E was opposite to that of the other 8 metabolites described in this paper and onnamide E did not show cytotoxic activity against the P388 cell line at a concentration of 0.4 μg/mL, indicating the importance of the conformation of the C11-C15 THP ring, the presence of an oxygen atom on C10, and/or the stereochemistry of C13 in the cytotoxic properties.

The NOESY spectrum of pseudoonnamide A, which gave similar cross-peaks all through the molecule, most notably cross-peaks between H7 and both of the C5 methylene protons, suggested that **8** had the same stereochemistry as onnamide A.[17] Handling of the onnamides gave rise to adverse skin reactions. This is hardly surprising in light of their structural relationship to pederin, the active principle of the blister beetle.[10b]

Experimental Section

Ultraviolet spectra were obtained in methanol solution on a Hitachi 330 spectrophotometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AM600 NMR spectrometer or a Bruker AC300 NMR spectrometer. ^1H NMR chemical shifts are reported with respect to CHD_2OD (δ =3.30) and ^{13}C NMR chemical shifts with respect to CD_3OD (δ = 49.0). FAB mass spectra were measured on a JEOL SX-102 mass spectrometer in positive ionization mode by using glycerol as the matrix.

Isolation of 1-9 The concentrated EtOH extract of the sponge (15 kg, wet weight) was partitioned between water and ether, followed by water and *n*-BuOH. The MeOH soluble portion (16.8 g) of the *n*-BuOH phase was chromatographed on a column of Sephadex LH-20 (5 x 80 cm) with MeOH. The 240-480 mL fraction (10.2 g) was subjected to ODS chromatography (70/230 mesh, 11 x 4 cm) eluting with MeOH/H_2O (3:7, 5:5, 10:0) and $CHCl_3$/MeOH/H_2O (7:3:0.5). The major cytotoxic material (2.3 g), eluted with MeOH/H_2O (5:5), was fractionated on an ODS column (70/230 mesh, 5 x 5 cm) with MeCN/H_2O (1:9, 2:8, and 4:6), MeOH, and $CHCl_3$/MeOH/H_2O (7:3:0.5). The fraction eluted with MeCN/H_2O (4:6) (yield 1.7 g) was chromatographed on a silica gel column (5 x 10 cm) with $CHCl_3$/MeOH/H_2O (8:2:0.15 and 7:3:0.5). The fraction eluted with $CHCl_3$/MeOH/H_2O (7:3:0.5) was purified by HPLC on TOSO ODS80TM (2.2 x 30 cm) and Capcell Pak C18 (2 x 25 cm) connected in a series with MeOH/H_2O (6:4) to yield **3** (90 mg) and **9** (330 mg). Fractions which turned out to be a mixture by ^1H NMR analysis were further purified by ODS-HPLC on Cosmosil 5C18AR (2 x 25 cm) with MeCN/H_2O (33:67, 4:6, or 45:55) to provide **1** (19 mg), **2** (6 mg), **4** (7 mg), and **5** (8 mg). A mixture of **6** and **7** was further separated by HPLC on YMC AM-ODS (2 x 25 cm) with *n*-PrOH/50 mM KH_2PO_4 (2:8) (yield, **6**, 2.6 mg; **7**, 1.3 mg). **Caution!** Onnamide-rich fractions cause adverse skin reactions.

Another specimen of *Theonella* sp. designated UTS5410 (1 kg) was collected off Hachijo Island by SCUBA (-15 m) and extracted with EtOH. The extract was partitioned between water and ether. The aqueous phase was separated by flash chromatography on ODS (5 x 5 cm; MeOH/H2O, 2:8, 5:5, 7:3, 9:1, 10:0 and $CHCl_3$/MeOH/H_2O, 7:3:0.5) followed by HPLC on Cosmosil 5C18AR (2 x 25 cm) with MeOH/H_2O (7:3) with 0.05 % TFA to yield **8** (1.5 mg).

13-Des-O-methyl-onnamide A (**1**): a glassy solid; $[\alpha]^{23}_D$ +70.0° (*c* 0.1, MeOH); HRFABMS (M + H)$^+$ *m/z* 780.4434 ($C_{38}H_{62}N_5O_{12}$, + 3.9 mmu); UV λmax (MeOH) 298 nm (ε 29000); ^1H NMR (CD_3OD) δ 7.14 (11.0, 15.0; H25), 6.52 (10.7, 14.8; H23), 6.26 (11.0, 14.8; H24), 6.21 (10.7, 14.8; H22), 6.06 (15.0; H26), 5.94 (7.0, 7.0, 14.8; H21), 5.81 (9.8; H10), 5.11 (7.0; 10-OCH), 4.81 (2.0, 2.0; 4=CH), 4.73 (7.0; 10-OCH), 4.64 (2.0, 2.0; 4=CH), 4.38 (5.2, 8.1; H2'), 4.22 (s; H7), 4.08 (6.6, 10.5; H12), 4.03 (6.6, 9.8; H11), 4.01 (10.4; H13), 3.87 (dq, 2.6, 6.8; H2), 3.65 (m; H17), 3.45 (4.1, 8.1; H15), 3.23 (3H, s; 6-OCH_3), 3.20 (2H, m; H_25'), 2.39 (2.0, 2.0, 14.3; H5), 2.33 (14.3; H5), 2.20 (m; H20), 2.19 (dq, 2.6, 6.8; H3), 2.14 (m; H20), 1.90 (m; H3'), 1.74 (m; H3'), 1.63 (2H, quint, 7.3; H_24'), 1.55 (m; H19), 1.53 (2H, m; $H_2$16), 1.48 (m; H18), 1.42 (m; H19), 1.30 (m; H18), 1.17 (3H, 6.8; 2-CH_3), 0.98 (3H, s; 14-CH_3), 0.96 (3H, 6.8; 3-CH_3), 0.88 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Dihydroonnamide A(**2**): a glassy solid; $[\alpha]^{23}_D$ +74.0° (*c* 0.2, MeOH); HRFABMS (M + H)$^+$ *m/z* 796.4775 ($C_{39}H_{66}N_5O_{12}$, + 6.7 mmu); UV λmax (MeOH) 260 nm (ε 22000); ^1H NMR (CD_3OD) δ 7.10 (10.8, 15.1; H25), 6.20 (10.8, 15.1; H24), 6.08 (7.1, 7.1, 15.1; H24), 6.00 (15.1; H26), 5.80 (9.3; H10), 5.20 (6.9; 10-OCH), 4.80 (6.9; 10-OCH), 4.80 (2.1, 2.1; 4=CH), 4.64 (2.1, 2.1; 4=CH), 4.36 (5.2, 7.6; H2'), 4.24 (s; H7), 4.16 (6.4, 8.1; H12), 3.97 (6.4, 9.3; H11), 3.88 (dq, 2.5, 6.6; H2), 3.64 (8.1; H13), 3.62 (m; H17), 3.55 (3H, s; 13-OCH_3), 3.49 (6, 7; H15), 3.24 (3H, s; 6-OCH_3), 3.22 (6.8, 6.8, 13.5; H5'), 3.18 (7.2, 7.2, 13.5; H5'), , 2.40 (2.1, 2.1, 14.3; H5), 2.32 (14.3; H5), 2.19 (dq, 2.5, 7.1; H3), 2.18 (2H, q, 7.1; H_222), 1.89 (m; H3'), 1.73 (m; H3'), 1.62 (2H, quint, 7.1; H_24'), 1.53 (2H, brt, 5.9; $H_2$16), 1.45 (4H, m; H18, H19, H_221), 1.38 (m; H20), 1.30 (3H, m; H18, H19, H20), 1.17 (3H, 6.6; 2-CH_3), 1.00 (3H, s; 14-CH_3), 0.96 (3H, 7.1; 3-CH_3), 0.86 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Onnamide B(**3**): a glassy solid; $[\alpha]^{23}_D$ +61.8° (*c* 0.5, MeOH); HRFABMS (M + H - MeOH)$^+$ *m/z* 736.4204 ($C_{36}H_{58}N_5O_{11}$, + 7.3 mmu); UV λmax (MeOH) 260 nm (ε 16000); ^1H NMR (CD_3OD) δ 7.10 (10.8, 15.1; H23), 6.24 (10.8, 15.0; H22), 6.10 (6.9, 6.9, 15.0; H21), 6.02 (15.1; H24), 5.79 (9.3; H10), 5.20 (6.9; 10-OCH), 4.79 (6.9; 10-OCH), 4.79 (2.2, 2.2; 4=CH), 4.63 (2.2, 2.2; 4=CH), 4.35 (5.1, 7.9; H2'), 4.23 (s; H7), 4.16 (6.5, 9.8; H12), 3.97 (6.5, 9.3; H11), 3.87 (dq, 2.8, 6.6; H2), 3.65 (m; H17), 3.63 (9.8; H13), 3.55 (3H, s; 13-OCH_3), 3.47 (3.1, 9.3; H15), 3.23 (3H, s; 6-OCH_3), 3.22 (6.8, 6.8, 13.6; H5'), 3.18 (7.2, 7.2, 13.6; H5'), 2.39 (2.2, 2.2, 14.3; H5), 2.31 (14.3; H5), 2.22 (m; H20), 2.19 (dq, 2.8, 7.0; H3), 2.15 (m; H20), 1.89 (m; H3'), 1.73 (m; H3'), 1.62 (2H, quint, 7.1; H_24'), 1.57 (m; H19), 1.53 (2H, m; $H_2$16), 1.49 (m; H18), 1.43 (m; H19), 1.29 (m; H18), 1.17 (3H, 6.6; 2-CH_3), 1.00 (3H, s; 14-CH_3), 0.95 (3H, 7.0; 3-CH_3), 0.85 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

17-Oxoonnamide B (**4**): a glassy solid; $[\alpha]^{23}_D$ +59.7° (*c* 0.2, MeOH); HRFABMS (M + H - MeOH)$^+$ *m/z* 734.4155 ($C_{36}H_{56}N_5O_{11}$, + 17.9 mmu); UV λmax (MeOH) 260 nm (ε 20000); ^{1}H NMR (CD_3OD) δ 7.09 (10.8, 15.2; H23), 6.21 (10.7, 15.0; H22), 6.04 (7.0, 7.0, 15.0; H21), 6.02 (15.2; H24), 5.74 (8.5; H10), 5.16 (6.9; 10-OCH), 4.79 (6.9; 10-OCH), 4.79 (2.2, 2.2; 4=CH), 4.63 (2.2, 2.2; 4=CH), 4.36 (5.3, 7.5; H2'), 4.28 (s; H7), 4.14 (6.0, 9.1; H12), 3.95 (3.0, 9.3; H15), 3.90 (dq, 2.6, 6.6; H2), 3.87 (6.0, 8.5; H11), 3.59 (brd, 9.1; H13), 3.55 (3H, s; 13-OCH_3), 3.24 (3H, s; 6-OCH_3), 3.23 (7.0, 7.0, 13.6; H5'), 3.18 (7.2, 7.2, 13.6; H5'), 2.53 (9.3, 16.0; H16), 2.47 (3.0, 16.0; H16), 2.47 (7.4, 7.4, 17.6; H18), 2.40 (7.2, 7.2, 17.6; H18), 2.37 (2.2, 2.2, 14.3; H5), 2.28 (14.3; H5), 2.19 (dq, 2.6, 7.0; H3), 2.14 (2H, brq, 7.5; $H_2$20), 1.89 (m; H3'), 1.73 (m; H3'), 1.64 (2H, m; $H_2$19), 1.62 (2H, m; $H_2$4'), 1.18 (3H, 6.6; 2-CH_3), 1.02 (3H, s; 14-CH_3), 0.94 (3H, 7.0; 3-CH_3), 0.86 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Onnamide C (**5**): a glassy solid; $[\alpha]^{23}_D$ +45.4° (*c* 0.2, MeOH); HRFABMS (M + H)$^+$ *m/z* 824.4342 ($C_{39}H_{62}N_5O_{14}$, + 4.9 mmu); UV λmax (MeOH) 282 nm (ε 16000); ^{1}H NMR (CD_3OD) δ 7.38 (11.4, 15.3; H24), 7.26 (11.4, 15.0; H25), 7.00 (15.3; H23), 6.54 (15.0; H26), 5.78 (9.2; H10), 5.20 (7.0; 10-OCH), 4.82 (2.1, 2.1; 4=CH), 4.80 (7.0; 10-OCH), 4.65 (2.1, 2.1; 4=CH), 4.37 (5.3, 7.5; H2'), 4.28 (s; H7), 4.15 (7.5, 9.8; H12), 4.04 (2.1, 3.5, 9.2, 11.7; H17), 3.95 (7.5, 9.2; H11), 3.88 (dq, 2.4, 6.6; H2), 3.63 (brd, 9.8; H13), 3.55 (3H, s; 13-OCH_3), 3.45 (1.8, 10.0; H15), 3.25 (3H, s; 6-OCH_3), 3.20 (2H, m; $H_2$5'), 2.41 (2.1, 2.1, 14.3; H5), 2.34 (14.3; H5), 2.19 (dq, 2.4, 7.0; H3), 1.90 (m; H3'), 1.88 (m; H19), 1.76 (m; H3'), 1.74 (3H, m; H18, H19, H20), 1.63 (2H, m; $H_2$4'), 1.60 (2H, m; H16, H20), 1.54 (1.8, 9.2, 14.0; H16), , 1.17 (3H, 6.6; 2-CH_3), 1.15 (m; H18), 1.00 (3H, s; 14-CH_3), 0.97 (3H, 7.0; 3-CH_3), 0.85 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Onnamide D (**6**): a glassy solid; $[\alpha]^{23}_D$ +51.4° (*c* 0.1, MeOH); HRFABMS (M + H - MeOH)$^+$ *m/z* 734.4367 ($C_{37}H_{60}N_5O_{10}$, + 2.7 mmu); UV λmax (MeOH) 298 nm (ε 24000); ^{1}H NMR (CD_3OD) δ 7.14 (11.2, 15.2; H25), 6.52 (10.7, 14.8; H23), 6.26 (11.2, 14.8; H24), 6.19 (10.7, 14.7; H22), 6.06 (15.2; H26), 5.93 (7.0, 7.0, 14.7; H21), 5.36 (7.6; H10), 4.80 (2.1, 2.1; 4=CH), 4.65 (2.1, 2.1; 4=CH), 4.37 (5.2, 7.5; H2'), 4.26 (s; H7), 3.92 (3.1, 6.9, 7.6; H11), 3.88 (dq, 2.8, 6.6; H2), 3.66 (m; H17), 3.59 (4.6, 10.5; H13), 3.44 (2.0, 10.3; H15), 3.37 (3H, s; 10-OCH_3), 3.25 (3H, s; 6-OCH_3), 3.22 (6.7, 6.7, 13.5; H5'), 3.17 (7.1, 7.1, 13.5; H5'), 2.49 (2.1, 2.1, 14.3; H5), 2.37 (14.3; H5), 2.20 (dq, 2.8, 7.0; H3), 2.16 (2H, brq, 6.9; $H_2$20), 1.98 (3.1, 4.6, 13.7; H12), 1.89 (m; H3'), 1.74 (6.9, 10.5, 13.7; H12), 1.73 (m; H3'), 1.63 (m; H16), 1.61 (2H, quint, 7.3; $H_2$4'), 1.58 (2H, m; H16, H19), 1.46 (2H, m; H18, H19), 1.37 (m; H18), 1.16 (3H, 6.6; 2-CH_3), 0.97 (3H, 7.0; 3-CH_3), 0.92 (3H, s; 14-CH_3), 0.85 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Onnamide E (**7**): a glassy solid; $[\alpha]^{23}_D$ +64.0° (*c* 0.05, MeOH); HRFABMS (M + H)$^+$ *m/z* 734.4336 ($C_{37}H_{60}N_5O_{10}$, - 0.4 mmu); UV λmax (MeOH) 299 nm (ε 14000); ^{1}H NMR (CD_3OD) δ 7.14 (11.3, 15.0; H25), 6.53 (10.6, 14.8; H23), 6.28 (11.3, 14.8; H24), 6.19 (10.6, 15.1; H22), 6.08 (15.0; H26), 5.89 (7.0, 7.0, 15.1; H21), 4.79 (2.0, 2.0; 4=CH), 4.63 (2.0, 2.0; 4=CH), 4.37 (5.4, 8.5; H2'), 4.21 (s; H7), 4.17 (3.5, 10.4; H15), 3.87 (dq, 2.6, 6.6; H2), 3.83 (m; H11), 3.63 (4.2, 8.5; H13), 3.52 (4.1, 13.7; H10), 3.23 (3H, s; 6-OCH_3), 3.20 (2H, m; $H_2$5'), 2.77 (10.4, 14.5; H16), 2.57 (3.5, 14.5; H16), 2.56 (7.1, 7.1, 17.6; H18), 2.49 (7.3, 7.3, 17.6; H18), 2.33 (2.0, 2.0, 14.3; H5), 2.22 (14.3; H5), 2.20 (dq, 2.6, 7.0; H3), 2.16 (2H, brq, 7.2; $H_2$20), 1.89 (m; H3'), 1.86 (m; H12), 1.73 (m; H3'), 1.70 (2H, quint, 7.3; $H_2$19), 1.62 (2H, quint, 7.1; $H_2$4'), 1.47 (8.5, 8.5, 13.5; H12), 1.17 (3H, 6.6; 2-CH_3), 0.98 (3H, s; 14-CH_3), 0.94 (3H, 7.0; 3-CH_3), 0.90 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

Pseudoonnamide A(**8**): a glassy solid; $[\alpha]^{23}_D$ +64° (*c* 0.05, MeOH); HRFABMS (M + H)$^+$ *m/z* 780.4463 ($C_{38}H_{62}N_5O_{12}$, + 6.8 mmu); UV λmax (MeOH) 298 nm (ε 26200); ^{1}H NMR (CD_3OD) δ 7.19 (11.2, 15.0; H25), 6.57 (10.7, 14.9; H23), 6.31 (11.2, 14.9; H24), 6.23 (10.7, 15.1; H22), 6.06 (15.0; H26), 5.97 (7.0, 7.0, 15.0; H21), 5.79 (9.6; H10), 5.22 (6.9; 10-OCH), 4.85 (2.1, 2.1; 4=CH), 4.79 (6.9; 10-OCH), 4.67 (2.1, 2.1; 4=CH), 4.46 (4.9, 8.1; H2'), 4.17 (6.6, 9.9; H12), 4.15 (dq, 2.9, 6.7; H2), 3.97 (6.8, 9.8; H11), 3.95 (s; H7), 3.67 (10.2; H13), 3.64 (m; H17), 3.57 (3H, s; 13-OCH_3), 3.45 (6.2, 6.2; H15), 3.22 (2H, m; $H_2$5'), 2.73 (2.1, 2.1, 13.8; H5), 2.18 (dq, 2.9, 7.0; H3), 2.18 (m; H20), 2.12 (m; H20), 2.10 (13.8; H5), 1.94 (m; H3'), 1.76 (m; H3'), 1.65 (2H, quint, 7.3; $H_2$4'), 1.58 (m; H19), 1.50 (2H, m; $H_2$16), 1.48 (m; H18), 1.42 (m; H19), 1.28 (m; H18), 1.08 (3H, 6.7; 2-CH_3), 1.00 (3H, 7.0; 3-CH_3), 0.99 (3H, s; 14-CH_3), 0.85 (3H, s; 14-CH_3); ^{13}C NMR (CD_3OD) see Table I.

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12. The H13 proton signal was broad in **2-5** and in **9** at 600 MHz. Broadening of the ^{13}C NMR signals for C11 and one of the methyls on C14 were reported for the mycalamides, truncated derivatives of onnamides at the bond between C18 and C19. Perry, N. B.; Blunt, J. W.; Munro, M. H. G.; Thompson, A. M. *J. Org. Chem.* **1990**, *55*, 223. 13-Des-*O*-methylonnamide A decomposed upon storage in CD_3OD at 4 °C for one month. From the degradation mixture, we could recover 20 % of **1**, 10 % each of a truncated derivative cleaved between C21 and C22, in which C21 was oxidized to an aldehyde, and the 21, 22-diol, indicating the formation of a 21, 22-dioxetane as an intermediate. Compounds **2-9** were stable in CD_3OD at 4 °C. At present we do not know the reason for the extreme sensitivity of **1** toward oxidation. Characterization of the degradation products of **1** and other metabolites, probably decomposition compounds of onnamide A, will be reported elsewhere.
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