Onnamide F: A New Nematocide from a Southern Australian Marine Sponge, *Trachycladus laevispirulifer*

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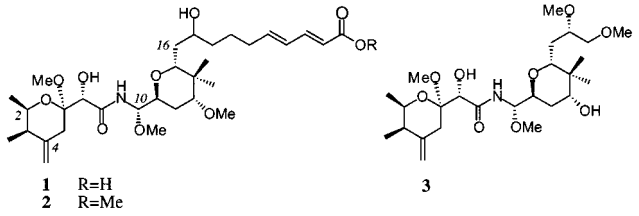
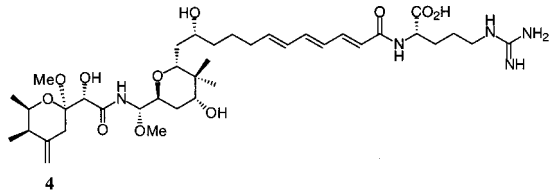
A southern Australian marine sponge, *Trachycladus laevispirulifer*, has yielded a potent new nematocide with antifungal activity which has been identified as onnamide F (**1**). The structure for **1** was assigned by detailed spectroscopic analysis and chemical conversion to the methyl ester **2**. Onnamide F contains a common structural motif previously described in a number of natural products exhibiting interesting pharmacological activities, including the insect chemical defense agent pederin (**3**), and the sponge metabolites the onnamides, mycalamides, and theopederins.

Our investigations into the chemistry of southern Australian marine organisms are aimed at the discovery of metabolites with potential as antiparasiticides. We are particularly interested in marine metabolites effective against commercially important parasitic nematodes. Recent examples of nematocidal agents uncovered during our investigations include an array of epoxylipids from the brown alga *Notheia anomala*[1] and the amphilactams A−D and geodin A Mg salt from sponges of the genera *Amphimedon*[2] and *Geodia*,[3] respectively, all collected from waters off the southern Australian coast. In this report we described the outcome of investigations into a specimen of *Trachycladus laevispirulifer* (Trachycladidae, Carter 1879) collected by hand (scuba) in Port Phillip Bay, Victoria. Bioassay-directed fractionation of the crude EtOH extract of this sponge yielded a potent nematocidal agent, to which we have assigned the structure and trivial name onnamide F (**1**).

The crude EtOH extract of a specimen *T. laevispirulifer* was found to be a potent inhibitor of larval development of the parasitic nematode *Haemonchus contortus* (LD_{99} 163 μg/mL) and inhibitor of the growth of the fungi *Saccharomyces cerevisae* (LD_{99} 82 μg/mL). The EtOH extract was decanted, concentrated in vacuo, and triturated with CH_2Cl_2, after which the soluble portion was fractionated by silica gel solid-phase extraction (eluting from hexanes to EtOAc, followed by MeOH flush). The MeOH flush from this fractionation yielded an unstable pale yellow solid with significant nematocidal (LD_{99} 5.2 μg/mL) activity. At high concentrations **1** inhibited larval development at the L1 larval stage, with a more delayed toxicity manifesting at the L2 stage observed at lower metabolite concentrations.[4] During the isolation procedure the nematocidal and antifungal activities were concurrently enriched, supporting the view that a common active component was responsible for both activities. The pale yellow solid **1** was active against *S. cerevisiae* with an LD_{99} value of 1.4 μg/mL. No activity was observed against *Bacillus subtilis* or *Eschericha coli*, indicating a selective toxicity for eukaryotes.

Initial ^{1}H NMR analysis revealed a conjugated diene and, given past experiences,[5] care was taken to quickly acquire spectroscopic data while avoiding unnecessary exposure to chromatographic media, intense light, heat, air, and pH variations. Although stored in the dark at <−20 °C when not required for spectroscopic analysis, samples of **1** did undergo gradual decomposition. This decomposition was accelerated by aprotic solvents (CH_2Cl_2, $CDCl_3$) and retarded, but not eliminated, by protic solvents (CH_3OH). In an effort to improve stability, the methyl ester **2** was prepared by esterification with diazomethane. Interestingly, the ester **2** was a more potent inhibitor of nematode development (LD_{99} 2.6 μg/mL) but less active against *S. cerevisiae* (LD_{99} 5.2 μg/mL).





ESI(+)MS analysis of **1** revealed a pseudomolecular ion (M + Na, Δmmu −3.4), consistent with a molecular formula $C_{31}H_{51}NO_{10}$, requiring seven double bond equivalents. Analysis of the NMR (CD_3OD) data for **1** revealed resonances consistent with two 3° methyls (^{1}H 0.86 and 0.97; ^{13}C 16.6 and 24.7 ppm), two 2° methyls (^{1}H 0.97 and 1.16; ^{13}C 12.4 and 18.2 ppm), three methoxy moieties (^{1}H 3.26, 3.38, 3.40; ^{13}C 48.7, 57.0, 57.7 ppm), two hydroxymethines (^{1}H 3.68, 4.28; ^{13}C 72.2, 74.4 ppm), two 1,2-disubstituted double bonds (^{1}H 5.80, 6.08, 6.22, 7.11; ^{13}C 124.5, 130.3, 143.4, 144.1 ppm), one 1,1-disubstituted double bond (^{1}H 4.65, 4.80; ^{13}C 148.3, 110 ppm), two acid/ester/amide carbonyls (^{13}C 174.8, 174.0 ppm), and a quaternary acetal carbon (^{13}C 101.3 ppm). These observations required that **1** be bicyclic. The presence of a carboxylic acid functionality was confirmed by methylation of **1** with

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Table 1. NMR (CD_3OD, 400 MHz) Data for Onnamide F (**1**)

	^{13}C δ (m)[a]	^{1}H δ (m, *J* Hz)	COSY (TOCSY)[c]	HMBC ($^{1}H-^{13}C$)
2	70.3 (d)	3.88 (dq, 2.4, 6.6)	2-Me, H-3 (*3-Me*)	3-Me
2-Me	18.2 (q)	1.16 (d, 6.6)	H-2 (*H-3*)	C-2, C-3
3	43.0 (s)	2.20 (m)	3-Me,H-2 (*2-Me*)	3-Me, C-4, 4-CH_2, C-5
3-Me	12.4 (q)	0.97 (d, 6.4)	H-3 (*H-2*)	C-2,C-3,C-4
4	148.3 (s)			
4-CH_{2a}	110.0 (t)	4.65 (d, 2.0)	Hb-5 (*4-CHb, Ha-5*)	C-3,C-5
4-CH_{2b}		4.80 (d, 2.0)	Hb-5 (*4-CHa, Ha-5*)	C-3, C-5
5_a	34.8 (t)	2.35 (d, 14.4)	Hb-5 (*4-CHa, 4-CHb*)	C-3, C-4, 4-CH_2, C-6, C-8
5_b		2.52 (ddd, 2.0, 2.0, 14.4)	Ha-5, 4-CHb, 4-CHa	C-4, 4-CH_2, C-6
6	101.3 (s)			
6-OMe	48.7 (q)	3.26 (s)		C-6
7	74.4 (d)	4.28 (s)		C-5, C-6
8	174.8 (s)			
9-NH[b]		7.17 (d, 10.4)	H-10	
10	81.7 (s)	5.28 (d, 5.8)	NH-9, H-11 (*Hb-12, H-13*)	C-8, 10-OMe, C-12
10-OMe	57.0 (q)	3.34 (s)		C-10
11	72.5 (d)	3.97 (ddd, 4.0, 5.8, 8.8)	H-10, Ha-12, Hb-12 (*H-13*)	C-10
12a	26.3 (t)	1.64 (m)	H-11, Hb-12, H-13	C-13
12_b		2.04 (m)	H-11, Ha-12, H-13 (*H-10*)	
13	82.7 (d)	3.14 (dd, 4.0, 8.8)	Ha-12, Hb-12 (*H-10, H-11*)	13-OMe
13-OMe	57.7 (q)	3.38 (s)		C-13
14	39.1 (s)			
14-Me_a	16.6 (q)	0.86 (s)		C-14, 14-Me_b, C-15
14-Me_b	24.7 (q)	0.97 (s)		C-14, 14-Me_a, C-15
15	81.6 (d)	3.49 (dd, 1.6, 10.8)	Ha-16, Hb-16 (*H-17*)	
16_a	37.4 (t)	1.52 (m)	H-15, Hb-16, H-17	
16_b		1.78 (m)	H-15, Ha-16, H-17	
17	72.2 (d)	3.68 (m)	Ha-16, Hb-16, H_2-18 (*H-15, H_2-20*)	
18	36.8 (t)	1.37 (m)	H-17	
19_a	25.9 (t)	1.47 (m)	(*H_2-20*)	
19_b		1.58 (m)	H_2-20	
20	33.8 (t)	2.20 (m)	Hb-19, H-21 (*H-17, Ha-19, H-22, H-24*)	C-19
21	143.4 (d)	6.16 (dd, 15.2, 7.6)	H_2-20, H-22	C-19, C-20, C-22, C-23
22	130.3 (d)	6.24 (dt, 10.8, 15.2)	H-21, H-23 (H_2-20, H-24)	
23	144.4 (d)	7.21 (dd, 10.8, 15.4)	H-22, H-24	C-21, C-22, C-25
24	124.5 (d)	5.78 (d, 15.4)	H-23 (*H_2-20, H-22*)	C-22
25	174.0 (s)			

[a] Supported by DEPT and gHMQC experiments. [b] NMR obtained in $CDCl_3$. [c] Additional TOCSY correlations in italics.

diazomethane to yield the methyl ester **2**, which displayed a characteristic NMR resonance (^{1}H δ 3.71).

Analysis of the spectroscopic data for **1** confirmed a close structural relationship with the bioactive metabolites known as pederin,[6−8] theopederins,[9,10] mycalamides,[11−14] and onnamides.[15−17] Pederin (**3**), the chemical defense agent of the blister beetle, was the first reported example of this structure class. The mycalamides, theopederins, and onnamides are sponge metabolites closely related to pederin. Most of these sponge metabolites feature a C-10/C-12 methylene dioxy bridge, which was not evident in **1**. The spectroscopic data reported for the O-1 to C-17 subunit in pederin (**3**) and onnamide D (**4**) were very close to that for **1**. The difference in ^{1}H and ^{13}C NMR (CD_3OD) chemical shifts between common substructures in **1** and **4** was $<\pm0.1$ and $<\pm0.6$ ppm, respectively, while ^{1}H NMR ($CDCl_3$) comparisons between **1** and **3** were even better ($\leq$0.07 ppm). Indeed, the only obvious difference across this subunit appeared to be the presence of a C-13 methoxy moiety in **1**, as opposed to a hydroxyl moiety in **3** and **4**. This was confirmed by a gHMBC correlation from H-13 (δ 3.14) to the 13-O*C*H_3 (57.7 ppm) (Table 1).

Furthermore, excellent comparisons between literature NMR data about the C-2 to C-15 subunit in **1** and onnamide D (**4**) (^{1}H and ^{13}C, CD_3OD), [15] and **1** and pederin (**3**) (^{1}H, $CDCl_3$),[8] supported a common relative stereochemistry about chiral centers within the submit. Given rotational freedom in the C-15 side chain, it was not possible to unambiguously correlate the relative stereochemistry about C-15 to that at C-17, and lack of material prevented an independent determination of absolute stereochemistry.

The remaining structural elements in **1** were attributed to an acyclic chain extending from C-17 and terminating in a dienoic acid functionality. All the structural assignments were supported by 2D NMR COSY, TOCSY, and gHMBC analysis (see Table 1).

In summary, the nematocidal agent present in this *T. laevispirulifer* was determined to be a close structure analogue of onnamide D (**4**) and was assigned the trivial name onnamide F[18] and structure as shown (**1**). Onnamide F (**1**) and its methyl ester **2** displayed potent in vitro nematocidal and antifungal activities and represent a new structural class of nematocides.

It is interesting to note that natural products incorporating the O-1 to C-18 pederin core (albeit with modest structural variation) have now been isolated from four genera of marine sponge, *Trachycladus*, *Mycale*, *Stylinos*, and *Theonella*, as well as the blister beetle *Paederus fuscipes*. While the genera *Mycale* and *Stylinos* belong to a common taxonomic grouping (family Mycalidae, order Poecilosclerida), this is not the case for either *Theonella* (family Theonellidae, order Lithisda) or *Trachycladus* (family Trachycladidae, order Hadromerida). That this rare and unusual structure class appears in such taxonomically distinct organisms may indicate a more complex, possibly microbial, biosynthetic origin.

On the matter of chemical ecology, pederin (**3**) is known to be a chemical defense agent for the blister beetle, and while not proven, the very bioactive onnamides, mycalamides, and theopederins could also function as defense allomones for their respective source sponges. This role would not be inconsistent with our observation of nema-

tocidal activity for this class of natural products, natural protection from parasites being a valuable evolutionary adaptation. Irrespective of their true biosynthetic origins, both terrestrial and marine examples of this structure class deserve ongoing consideration as antiparasitic agents.

Experimental Section

General Experimental Procedures. See ref 19.

Animal Material. A specimen of *Trachycladus laevispirulifer* (Museum of Victoria Registry Number F80006) was collected in April 1995 by scuba (−15 m) in Port Phillip Bay, Victoria, Australia (34° 02 S; 114° 44 E). A description of the specimen is as follows: growth form stalked, digitate-club-shaped branches (8 mm) roughly cylindrical, laterally coalescent; color on deck bright red-orange, color in EtOH pale pink; texture spongy, difficult to tear with a very tough stalk; oscules not seen; surface opaque, irregular, rugo-tuberculose, glossy; spicules megascleres oxeas and strongyles hastate, mucronate (310−450 × 4−10 μm); microscleres sigmaspires (10−12 μm), microrhabds (6−15 × 1−3 μm); ectosome a distinct crust of sigmaspires (1 mm); choanosome a densely spiculose axis of oxeas/strongyles becoming plumose, multispicular tracts extra-axially, cored by 2−5 megascleres, spongin visible, occasionally protruding the surface; spiroscleres dense throughout and megascleres scattered interstitially in the subectosome; collagen light. The sponge was transported to the laboratory, where it was documented, diced, and steeped in EtOH at −20 °C, prior to chemical analysis.

Extraction and Isolation. The EtOH extract of the specimen of *T. laevispirulifer* was decanted and concentrated in vacuo to yield a bright orange solid (36 mg) that was triturated with CH_2Cl_2. The CH_2Cl_2-soluble material was concentrated and the residue (9 mg) subjected to silica solid-phase extraction (10% stepwise elution of hexanes to EtOAc, with a MeOH flush). Elution with MeOH yielded onnamide F (**1**) (4 mg, 0.3% dry weight of sponge).

Onnamide F (1): pale yellow solid; $[\alpha]^{22}_D$ +22° (*c* 0.16%, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESI(+)MS *m/z* $(M + Na)^+$ 620.3377 (calcd for $C_{31}H_{51}NO_{10}Na$, 620.3411).

Onnamide F Methyl Ester (2). To a solution of onnamide F (**1**) (4 mg) in CH_2Cl_2 (5 mL) at 5 °C was added an ethereal solution of diazomethane until the reaction liquor turned a pale yellow color. The mixture was stirred for 1 h, after which the solvent was removed in vacuo to yield the methyl ester **2** (4 mg, 100%) as a pale yellow solid; $[\alpha]^{22}_D$ +24° (*c* 0.16, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (dd, J = 9.8 and 15.3 Hz, H-23), 6.27−6.17 (m, H-21 and H-22), 5.82 (d, J = 15.3 Hz, H-24), 5.28 (d, J = 5.8 Hz, H-10), 4.80 (d, J = 1.8 Hz, 4-CHb), 4.65 (d, J = 1.8 Hz, 4-CHa), 4.27 (s, H-7), 3.97 (ddd, J = 4.0, 5.8, 8.0 Hz, H-11), 3.88 (dq, J = 2.4 and 6.4 Hz, H-2), 3.71 (s, 25-OMe), 3.68 (m, H-17), 3.49 (dd, J = 1.6 and 11.2, Hz, H-15), 3.38 (s, 13-OMe), 3.34 (s, 10-OMe), 3.26 (s, 6-OMe), 3.14 (dd, J = 4.0 and 8.0 Hz, H-13), 2.52 (ddd, J = 1.8, 1.8 and 14.4 Hz, Hb-5), 2.35 (d, J = 14.4 Hz, Ha-5), 2.21 (m, H-3 and H-20), 2.05 (m, Hb-12), 1.78 (m, Hb-16), 1.64 (m, Ha-12), 1.58 (m, Hb-19), 1.52 (m, Ha-16), 1.47 (m, Ha-19), 1.37 (m, H-18), 1.16 (d, J = 6.4 Hz, 2-Me), 0.97 (s, 14-Me), 0.96 (d, J = 6.4 Hz, 3-Me), 0.86 (s, 14-Me); HRESI(+)MS $(M + Na)^+$ *m/z* 634.3579 (calcd for $C_{32}H_{53}NO_{10}Na$, 634.3567).

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