A Serendipitous Discovery of Isomotuporin-Containing Sponge Populations of *Theonella swinhoei*

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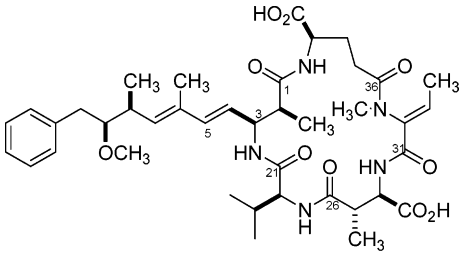
An in-depth LCMS examination of 14 different collections of Indo-Pacific *Theonella swinhoei* sponges resulted in the discovery of four diastereomeric analogues of the cyclic pentapeptide motuporin. These motuporin analogues all contain a novel 2*R* configuration for the Adda amino acid. Additionally, one analogue has a unique nonoxygenated Adda amino acid. In all, 15 different compounds were observed by LCMS or isolated. The stereochemistries of the constituent amino acids were determined through a combination of the advanced Marfey technique and ^{1}H NMR data.

The sustained worldwide interest over the past 25 years in the chemistry and biology of the sponge *Theonella swinhoei* (Lithistida, Theonellidae) is astonishing. Kashman was among the first, in the early 1980s, to recognize the potential of this sponge as a source of diverse secondary metabolites with reports on unique sterols[1] and complex polyketides headed by swinholide A.[2] This remarkably prolific species continues to be a source of powerfully bioactive substances, with motuporin (**1**)[3] being an early example. Currently, the biosynthetic products of *T. swinhoei* represent more than nine biosynthetic classes[4−12] and have been reported from diverse panoceanic locals including Papua New Guinea, Indonesia, the Philippines, Palau, the Red Sea, Japan, and Mozambique.



motuporin (**1**)

Careful examination of the literature along with scanning of our sponge repository indicated there were at least three phenotypes[13] of *T. swinhoei* with features shown in Figure 1. The first phenotype is defined by a characteristic red-purple ectosome and a cream-colored endosome, and more than 15 such samples have been examined by our group.[14] We found that the major constituents varied among the swinholides, motuporin or theonellapeptolide Id, but not all compounds were present in all collections. The second also possesses a red-purple ectosome but with a yellow to orange interior. Fusetani[13b] has indicated there are eight molecular frameworks isolable from such specimens including polytheonamides, cyclotheonamides, nuzumamide A, pseudotheonamides, onnamides, theopederins, orbiculamide A, and the aurantosides. Recently, both the UCSC lab and the Ireland group[13c] have observed aurantosides from the third phenotype, which is red-orange throughout. The preceding scenario represents a confounding circumstance that stimulated the research pursued in this study. However, it must be underscored that attempts to gain an understanding of the changing chemical signatures of *T. swinhoei* are inherently problematic because of the complex microbial communities that are always associated with it.[15]

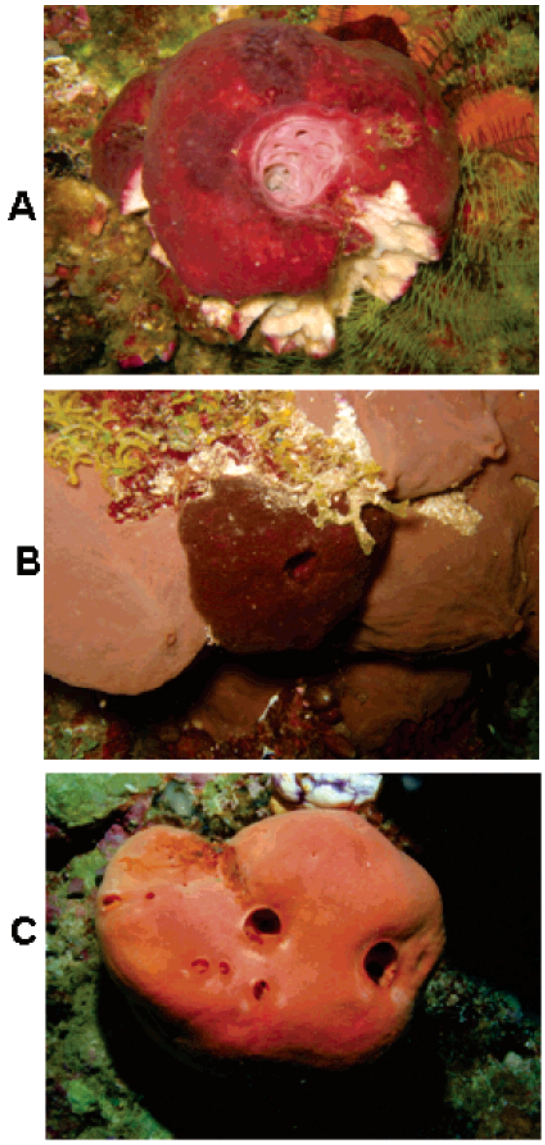


Figure 1. The three *Theonella swinhoei* phenotypes: (A) type I; (B) type II; and (C) type III.

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Table 1. *Theonella swinhoei* Collections and Their Respective Secondary Metabolites[a]

	collection numbers/location[b]													
compound	94590 Indo	94647 Indo	94648 Indo	00342 PNG	00356 PNG	00365 PNG	00399 PNG	02141 PNG	02142 PNG	03506 PNG	05221 PNG	05254 PNG	05259A PNG	05259B PNG
swinholides A, F	•		•	•	•	•								
swinholides B, C, D, G	•													
swinholide E		•											•	
swinhoeiamide A							•				•	•	•	•
theonellamine B	•			•	•					•				
theonellamide A			•			•							•	
papuamide D													•	
theopalauamide	•			•		•	•	•	•				•	
misakinolide A													•	
theoneberine							•						•	
theopederin J						•							•	
motuporin														
isomotuporin								•	•					

[a] Structures of named compounds and above and/or underwater photographs of all the sponges studied can bo found in Figures S8 and S9, respectively. [b]Indo = Indonesia, PNG = Papua New Guinea.

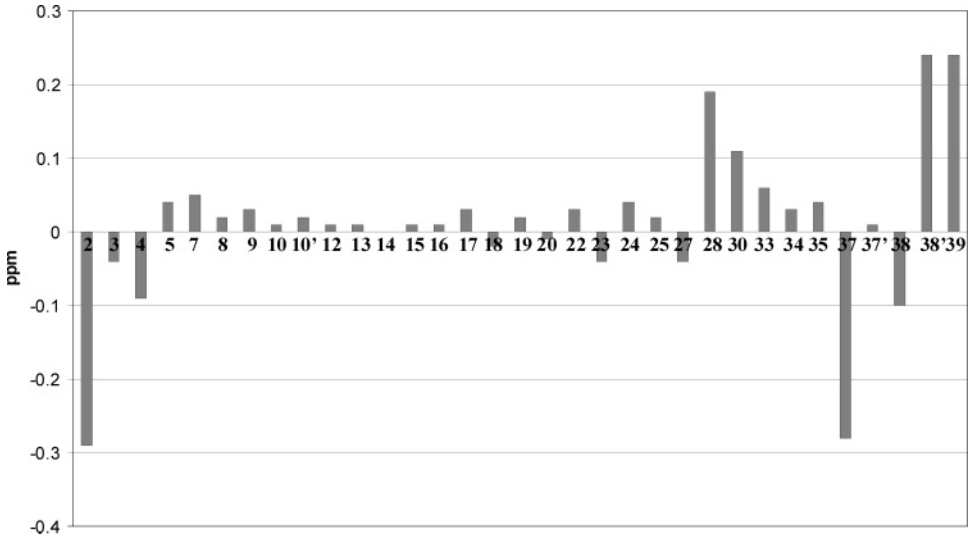
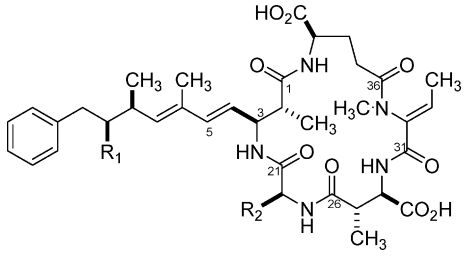


Figure 2. ^{1}H NMR chemical shift differences of motuporin (**1**)[3] versus isomotuporin (**2**).

We believed that a careful screening study by LCMS of Indo-Pacific-derived *T. swinhoei* could allow refinement of observations published in 1998.[14] In contrast to the previous study, we sought definition of both major and minor components. Another goal was to be able to reliably locate populations that contained motuporin (**1**) or the swinholides or both because there is continuing speculation that these compounds could be arising from associated cyanobacteria.[16] None of the 17 specimens[14] we previously examined contained the swinholides, yet **1** was present in 14 of them. Thirteen new collections of *T. swinhoei* were investigated and to our surprise none of them contained **1**, but two were a source of a motuporin diastereomer. This new compound, isomotuporin (**2**), along with three congeners, isomotuporins B (**3**), C (**4**), and D (**5**), plus 11 known compounds were either observed or isolated, and these results are described next.



*iso*motuporin (**2**): $R_1 = OCH_3$, $R_2 = CH(CH_3)_2$

*iso*motuporin B (**3**): $R_1 = OCH3$, $R_2 = CH(CH_3)CH_2CH_3$

*iso*motuporin C (**4**): $R_1 = OCH_3$, $R_2 = CH_2CH_3$

*iso*motuporin D (**5**): $R_1 = H$, $R_2 = CH(CH_3)_2$

Results and Discussion

The extracts of 14 *T. swinhoei* collections, obtained from different Indo-Pacific sites, were processed as described in the Experimental Section and then screened using LCMS. One of these (coll. no. 94590), though previously discussed,[14] was not evaluated by the methods used herein. There were 14 compounds profiled as shown in Table 1. Comparisons of our observed MS *m*/*z* values, UV curves, and diagnostic ^{1}H NMR shifts to those in the literature were used to dereplicate compounds but not their stereochemical details, including swinholide A[2] or F;[17] swinholide B,[17b] C,[17b] D,[17] or G;[17] swinholide E;[17] swinhoeiamide A;[7] theonellamine B;[18] theonellamide A;[19] papuamide D;[9] theopaluamide;[10] misakinolide A;[20] theoneberine;[21] and theopederin J[11] (see Figure S8). Only the planar structures of these compounds were determined, as their optical properties were not explored. Two of the samples (coll. nos. 02141 and 02142, 0.5 kg wet weight each) contained ESI $[M + H]^+$ *m*/*z* at 768.4 amu, diagnostic of motuporin (**1**). Purification of this

Table 2. NMR Data[a] for Compound **2** in MeOD-d_4

position	δ_C	δ_N[b]	δ_H (J in Hz)	gCOSY	gHMBC	NOE	^{13}N-HMBC
ADDA							
1	176.1						
2	45.4		2.73 dq (10.7, 7.0)	3, 17	1, 3, 17	3, 17	
3	56.2		4.57 dd (9.0, 10.7)	2, 4	2, 4, 5	2, 4, 5, 17	
4	126.2		5.52 dd (9.0, 15.5)	3, 5	3, 6	3, 5, 17, 18	
5	139.4		6.26 d (15.5)	4	3, 6, 7, 18	3, 4, 7, 17, 19	
6	133.9						
7	137.4		5.44 d (10.0)	8, 18	5, 8, 9, 18	5, 8, 9, 10a, 10b, 19	
8	37.9		2.59 d pent (10.0, 6.7)	7, 9, 19	6, 7, 9, 10, 19	18, 19	
9	88.5		3.26 m	8, 10a, 10b	7, 10, 11, 19, 20	Ar, 7, 19	
10a	39.1		2.82 dd (5.0, 14.1)	9, 10b	8, 9, 11, 12, 16	12, 16, 19	
10b			2.67 dd (7.3, 14.1)	9, 10a	8, 9, 11, 12, 16	12, 16	
11	140.6						
12	130.6		7.18 m		10, 14, 16	10a, 10b, 18, 19, 20	
13	129.3		7.24 m		11, 15		
14	127.1		7.17 m		12, 16		
15	129.3		7.24 m		11, 13		
16	130.6		7.18 m		10, 12, 14	10a, 10b, 18, 19, 20	
17	16.4		1.05 d (7.0)	2	1, 2, 3	2, 17	
18	13.0		1.60 d (1.1)	7	5, 6, 7	8, 12, 16, 19, 20	
19	16.7		1.01 d (6.7)	8	7, 8, 9	8, 10a, 12, 16, 18	
20	58.9		3.22 s		9	12, 16, 18	
Val							
21	171.3						
22	58.1		4.43 d (4.1)	23, 22NH	21, 23, 24, 26	23, 24, 25	22-N
23	30.1		2.45 m	22, 24, 25		22, 24, 25	22-N
24	17.0		0.80 d (7.1)	23	22, 23, 25	22, 23	
25	19.9		0.86 d (7.1)	23	22, 23, 24	22, 23	
22-N		119.5	8.32 d (10.2)	22			
βMeASP							
26	176.8						
27	39.8		3.19 dq (2.5, 7.0)	28, 30	26/29	28, 30	28-N
28	57.7		4.39 d (2.5)	27	27, 29, 30	27, 30	
29	176.7						
30	16.9		1.31 d (7.0)	27	26, 27, 28	27, 28	
28-N		110.6	8.89 d (6.8)				
NMeΔBUT							
31	165.7						
32	137.2						
33	138.1		7.00 q (7.1)	34	31, 32, 34		32-N
34	13.4		1.77 d (7.1)	33	31, 32	35	
35	35.2		3.11 s		32, 36	34	32-N
32-N		112.5					
GLU							
36	174.3						
37a	29.5		2.45 m	37b, 38a, 38b			
37b			2.05 m	37a, 38a			
38a	27.8		2.17 m	38b, 39, 37a			39-N
38b			2.05 m	38a, 39			
39	52.0		4.69 dd (3.9, 5.7)	38a, 38b		38a, 38b/37b	
40	174.6						
39-N		122.2					

[a] Measured at 600 MHz (^{1}H) (also see Figure S4) and 125 MHz (^{13}C). [b] NH_3 used as $\delta = 0$ reference.

compound followed by ^{1}H NMR analysis indicated variations in seven chemical shifts, indicating that a new diastereomer, isomotuporin (**2**), was in hand (Figure S1). The substantial differences (>0.1 ppm) in the shifts for seven protons can easily be seen by the plot in Figure 2. Alternatively, a similar comparison of the ^{1}H NMR data of natural **1**[3] versus synthetic[22] demonstrated that no shifts differed by more than 0.05 ppm. Further examination of the LCMS profiles for these two sponges indicated the presence of small amounts of three other motuporin analogues, B−D (as indicated by *m*/*z* values of 782.4, 754.4, and 738.4, respectively). Repeated semipreparative reversed-phase HPLC was used to purify these compounds as amorphous, white solids from the methanol and dichloromethane extracts.

The confirmation that isomotuporin (**2**), of molecular formula $C_{40}H_{57}N_5O_{10}$ by HRESIMS (*m*/*z* 768.4122 $[M + H]^+$), possessed the planar structure shown came from diagnostic 2D NMR correlations including gCOSY, gHMQC, gHMBC, and ^{15}N-HMBC (see Table 2 and Figure S1). While the ^{13}C NMR shifts of **1** and **2** were not very different, the ^{1}H NMR data of Figure 2 along with the observed rotation for **2** ($[\alpha]^{25}_D = -38.4$) versus that in the literature for **1** ($[\alpha]^{25}_D = -83.8$)[3] represented distinctive properties. The stereochemistry at the eight chiral centers (2*S*, 3*S*, 8*S*, 9*S*, 22*S*, 27*S*, 28*R*, 39*R*) and geometry across the three double bonds (4*E*, 6*E*, 32*Z*) have been rigorously proven for **1**.[3,22] A systematic approach was employed to evaluate these features in **2**. The identical vinylic proton shift values and coupling constants for **2** versus **1** confirmed the 4*E* and 6*E* geometries of the former. In addition, a strong NOE from Me-34 to Me-35 confirmed a 32*Z* geometry.

Subjecting **2** to acid hydrolysis (6 N HCl, 110 °C, 16 h), derivatization with Marfey's reagent,[23] and subsequent HPLC analysis was used to identify *S*-valine and *R*-glutamic acid by comparison with standard samples,[24] while the advanced Marfey technique[25] (Figure S2) was used to identify 3*S*-Adda and *R*-β-methyl aspartic acid. These data justified 3*S*, 22*S*, 28*R*, and 39R assignments. An *R*-erythro-β-methyl aspartic acid was assigned on the basis of the identical H-27 to H-28 *J*'s and δ'*s* observed between **1** and **2**, thereby indicating a 27*S* configuration. Likewise, identical 8*S*, 9*S* configurations for **2** versus **1** were deduced on the basis of

Table 3. NMR Data[a] for Compounds **3**−**5** in MeOD-d_4

	3		**4**		**5**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
ADDA						
1	176.0		176.1		176.1	
2	45.1	2.77 dq (10.7, 6.7)	45.3	2.71 dq (10.7, 6.9)	45.4	2.73 dq (10.7, 6.7)
3	55.9	4.58 dd (9.3, 10.5)	56.2	4.56 dd (9.1, 10.7)	56.1	4.58 dd (8.9, 10.6)
4	126.3	5.53 dd (8.8, 15.5)	126.4	5.47 dd (9.1, 15.4)	125.7	5.51 dd (8.9, 15.6)
5	139.2	6.26 d (15.5)	139.4	6.25 d (15.4)	139.6	6.28 d (15.7)
6	133.9		133.9		133.8	
7	137.1	5.43 d (9.8)	137.4	5.43 d (10.1)	140.9	5.34 d (9.5)
8	37.7	2.59 ddq (6.6, 10.0, 6.7)	37.9	2.59 ddq (6.9, 10.1, 6.8)	33.4	2.49 m
9	88.5	3.26 m	88.5	3.26 m	40.6	1.68 m
						1.54 m
10a	39.0	2.82 dd (4.8, 13.8)	39.2	2.82 dd (4.7, 13.9)	35.0	2.55 m
10b		2.67 dd (7.2, 14.1)		2.67 dd (7.3, 13.9)		
11	140.6		140.6		140.4	
12	130.6	7.19 m	130.6	7.19 m	129.5	7.13 m
13	129.3	7.25 m	129.4	7.25 m	129.4	7.23 m
14	127.1	7.18 m	127.3	7.18 m	126.8	7.12 m
15	129.3	7.25 m	129.4	7.25 m	129.4	7.23 m
16	130.6	7.19 m	130.6	7.19 m	129.5	7.13 m
17	16.4	1.04 d (6.9)	16.6	1.05 d (6.9)	16.4	1.05 d (6.9)
18	13.0	1.61 d (1.2)	13.2	1.61 d (1.2)	13.1	1.67 d (1.0)
19	16.4	1.01 d (6.7)	16.7	1.01 d (6.8)	21.3	0.98 d (6.7)
20	58.8	3.24 s	58.9	3.24 s		
	Aba		Ile		Val	
21	n/d		n/d		n/d	
22	58.3	4.37 d (2.4)	54.8	4.34 dd (3.6, 10.5)	58.2	4.44 dd (4.1, 9.6)
23	29.4	2.51 m	25.4	2.04 m	30.1	2.44 m
				1.43 ddq (5.6, 10.5, 7.5)		
24	12.6	0.91 d (6.7)	10.9	0.86 t (7.5)	16.8	0.80 d (6.9)
25	23.8	1.33 m			19.8	0.86 d (6.9)
25-Me	14.5	0.86 t (7.0)				
βMeASP						
26	176.9[b]		176.8[b]		177.5[b]	
27	40.0	3.18 m	40.5	3.04 dq (1.9, 6.8)	39.7	3.18 m
28	58.3	4.44 d (3.8)	57.9	4.41 d (1.9)	57.7	4.39 dd (2.6, 6.9)
29	176.7[b]		176.7[b]		176.7[b]	
30	16.7	1.31 d (6.9)	16.9	1.31 d (6.8)	16.8	1.30 d (6.9)
NMeΔBUT						
31	165.6		165.8		165.7	
32	137.3		137.1		137.2	
33	137.8	6.99 q (7.2)	138.1	7.00 q (7.1)	138.1	7.00 q (7.1)
34	13.4	1.77 d (7.1)	13.5	1.77 d (7.1)	13.4	1.77 d (7.1)
35	35.2	3.10 s	35.4	3.11 s	35.2	3.11 s
GLU						
36	173.6		174.4		174.2	
37a	29.4	2.53 m	29.6	2.47 m	29.4	2.46 m
37b		2.05 m		2.06 m		2.05 m
38a	27.7	2.18 m	28.0	2.17 m	27.8	2.18 m
38b		2.05 m		2.05 m		2.05 m
39	52.5	4.67 dd (4.1, 5.7)	52.1	4.70 dd (3.4, 5.4)	52.1	4.69 dd (3.8, 5.7)
40	n/d		n/d		n/d	

[a] Measured at 600 MHz (^{1}H) (also see Figures S5−S7) and 125 MHz (^{13}C). [b]Interchangeable; n/d = not detected; Aba = 2-aminobutyric acid.

their identical ^{1}H NMR data at these sites. Finally, by default the C-2 configuration of Adda was considered to be *R* since all other stereocenters and double-bond geometries in **2** were determined by the above analysis to be identical to those of **1**. Also supporting this assignment was that the largest ^{1}H NMR shift difference between **2** and **1** was at H-2 (Δ = 0.29 ppm, Figure 2). It is important to note that the shift differences shown in Figure 2 for **2** versus **1** at H-37 to H-39 accompanied by differences in $^3J_{38-39}$, and changes at H-28 and H-30 also accompanied by slight $^3J_{27-28}$ differences, appear due to local conformational variations. Therefore, only the 2*R* configuration of **1** was inverted to 2*S* in **2**.

The NMR properties of **2**, shown in Table 2, served as an important reference point to enable a concise approach to establishing the structures of the other three related congeners. Thus, isomotuporin B (**3**) ($[\alpha]^{25}_D = -29.7$), with a molecular formula of $C_{41}H_{59}N_5O_{10}$ established by HRESIMS (*m*/*z* 782.4358 $[M + H]^+$), differed from **2** by +14 amu. The addition of a $-CH_2-$ unit was easily rationalized by observing signals of a *sec*-butyl group evident from the data in Table 3, consisting of a methyl triplet at δ 0.86 (J = 7.0 Hz), a methylene at δ 1.33 (m), and a methyl doublet at δ 0.91 (J = 6.7 Hz), which are not present in **1** or **2**. Analysis of gHMBC data showed that in **3** an isoleucine side chain was in place of the valine side chain originally found in motuporin. All of the remaining 2D NMR data were unchanged for **3** versus **2**, supporting the planar structure shown. Similar logic was used to establish the gross structure for **4** of molecular formula $C_{39}H_{55}N_5O_{10}$ established by HRESIMS (*m*/*z* 754.4090 $[M + H]^+$). In this case there was a −14 amu difference relative to **2**, indicating the loss of a $-CH_2-$ unit. The aliphatic methyl region in **3** also exhibited a methyl triplet at δ 0.86 (J = 7.5 Hz); however, analysis of gHMBC data showed that in **4** a C-22 ethyl group replaced the C-22 isopropyl group of **2**.

Slightly greater differences were observed when comparing the properties of isomotuporin C (**5**) ($[\alpha]^{25}_D = -13.2$) with those of **2**.

The molecular formula of $C_{39}H_{55}N_5O_9$ for **5** was established by HRESIMS (*m*/*z* 738.4023 $[M + H]^+$). This differs from that of **2** by −30 amu, or a loss of an OCH_2 residue. It was immediately evident that the C-9 OCH_3 was replaced by an H because (a) no singlet was observed at δ 3.01, (b) the H-10 protons (δ 2.55 m) were isochronous, and (c) the DEPT spectrum showed C-9 (δ 40.6) was a CH_2 group. Finally, analysis of gHMBC data confirmed that a C_2H_4 group connected the aryl ring to C-8. The absolute stereochemistry deduced here for **2** was provisionally assigned to **3**−**5**. This is based on the parallel ^{1}H chemical shifts and coupling constants shown for this set and the supposition that a parallel biosynthetic gene cluster was involved in the production of these metabolites.

Conclusions

Some of the findings outlined above will be relevant to those seeking further understanding of factors that control PKS-mediated production of the swinholides and mixed PKS−NRPS elaboration of motuporin. The selection of the proper sponge phenotype using the natural history information of Figure 1 is an essential first step to probe for the swinholide or motuporin pathways. To date, no populations of this sponge have been identified that have both pathways simultaneously expressed. Until now all compounds known containing the Adda amino acid, including (−)-nodularin and microcystin-LR (both of cyanobacterial origin) and motuporin (**1**), carried a consistent 2*S* stereochemistry. The discovery of distinct sponge populations that produce either 2*S*-motuporin (**1**) or 2*R*-motuporin (**2**) is significant. Additionally, the loss of the C-9 OCH_3 in **5** is the first report of such a variation on the Adda side chain from natural sources. The results herein should now provide the impetus to stimulate further investigations on the ecology and molecular genetics of *T. swinhoei*.

Experimental Section

General Experimental Procedures. These are according to those previously published,[26] except ^{1}H NMR spectra were recorded on a Varian Inova 600 spectrometer operating at 599.8 MHz.

Animal Material. Specimens of *Theonella swinhoei* were collected in the waters near the following locations: coll. no. 94590, depth 90 ft, Sangihe Island, Indonesia; coll. nos. 94647 and 94648, Indonesia; coll. nos. 00342 and 00356, depth 60 ft, New Britain, Papua New Guinea; coll. nos. 00365 and 00399, depth 50 ft, New Britain, Papua New Guinea; coll. nos. 02141 and 02142, depth 50 ft, Normanby, Papua New Guinea; coll. no. 03506, depth 50 ft, Milne Bay, Papua New Guinea; coll. no. 05221, depth 50 ft, Rabaul and New Ireland, Papua New Guinea; coll. no. 05254, depth 50 ft, New Ireland, Papua New Guinea; coll. nos. 05259A and 05259B (blanched specimen), depth 55 ft, New Ireland, Papua New Guinea. Photographs of the sponges are available from the Crews laboratory.

Extraction and Isolation. All sponges were immediately preserved after collection according to our standard procedure[14] and transported back to the laboratory at ambient temperature. Analytical reversed-phase LCMS using a gradient of 10:90 CH_3CN/H_2O to 100% CH_3CN over 35 min was performed on organic solvent partition extracts[14] (coll. nos. 94590, 94647, 94648, 00342, 00356, and 00399) and ASE solvent extracts[26] (coll. nos. 02141, 02142, 03506, 05221, 05254, 05259A, and 05259B). The LCMS profiles of these extracts exhibited molecular ions (see Table 1) attributable to swinholide A (*m*/*z* 1389.8 $[M + H]^+$), swinholides B, C, or G (*m*/*z* 1375.9 $[M + H]^+$), swinholide E (*m*/*z* 1405.8 $[M + H]^+$), swinhoeiamide A (*m*/*z* 797.5 $[M + H]^+$), theonellamine B (*m*/*z* 1405.0 $[M + H]^+$), theonellamide A (*m*/*z* 1765.4 $[M + H]^+$), papuamide D (*m*/*z* 1385.7 $[M + H]^+$), theopalauamide (*m*/*z* 1750.5 $[M + H]^+$), misakinolide A (*m*/*z* 1338.0 $[M + H]^+$), theoneberine (*m*/*z* 779.8 $[M + H]^+$), theopederin J (*m*/*z* 626.4 $[M + H]^+$), and motuporin/isomotuporin (*m*/*z* 768.4 $[M + H]^+$). After ASE extraction of coll. no. 02142, the CH_3OH extract was subjected to preparative RP HPLC (20:80 CH_3CN/H_2O to 100% CH_3CN) to yield 11 fractions. Fractions 8−11 were further purified using semipreparative RP HPLC (65:35 CH_3CN/H_2O to 80:20 CH_3CN/H_2O) to yield **2** (10.4 mg), **3** (2.0 mg), **4** (2.2 mg), and **5** (3.0 mg).

Isomotuporin (2): colorless solid, $[\alpha]^{25}_D$ −38.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.48) nm; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 768.4122 $[M + H]^+$ (calcd for $C_{40}H_{57}N_5O_{10}$ + H, 768.4105).

Isomotuporin B (3): colorless solid, $[\alpha]^{25}_D$ −29.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.48) nm; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 782.4358 $[M + H]^+$ (calcd for $C_{41}H_{59}N_5O_{10}$ + H, 782.4335).

Isomotuporin C (4): colorless solid, $[\alpha]^{25}_D$ −21.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.48) nm; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 754.4090 $[M + H]^+$ (calcd for $C_{39}H_{55}N_5O_{10}$ + H, 754.4022).

Isomotuporin D (5): colorless solid, $[\alpha]^{25}_D$ −13.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.48) nm; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 738.4023 $[M + H]^+$ (calcd for $C_{39}H_{55}N_5O_9$ + H, 738.4073).

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Supporting Information Available: ^{1}H NMR comparison of motuporin (**1**) and isomotuporin (**2**), representation of gCOSY, gHMBC, and ^{15}N-HMBC correlations for isomotuporin (**2**), advanced Marfey analysis chromatograms, ^{1}H NMR spectra for **2**−**5**, and structures for compounds in Table 1. Above and underwater *Theonella* sponges and photographs of all of the sponges studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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