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Motuporin, A Potent Protein Phosphatase Inhibitor Isolated from the Papua New Guinea Sponge *Theonella swinhoei* Gray

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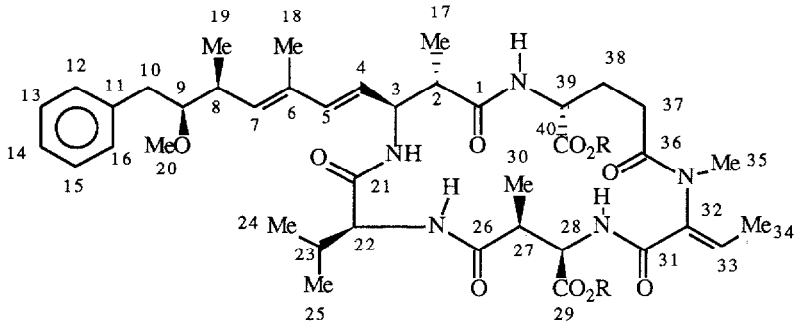
Abstract: *Motuporin (**1**), a cyclic pentapeptide that is a potent protein phosphatase-1 inhibitor and cytotoxin, has been isolated from the marine sponge Theonella swinhoei collected in Papua New Guinea. The structure of motuporin was elucidated by spectroscopic analysis and chemical degradation.*

Marine sponges belonging to the genus *Theonella* are proving to be a rich source of biologically active cyclic peptides that usually contain at least one previously unknown amino acid. The *Theonella* peptides reported to date include; orbiculamide A,[1] keramamides B to D,[2] cyclothoenamides A and B,[3] theonellamide F,[4] the theonellopeptolides,[5] and theonellamine B.[6] As part of an ongoing investigation of bioactive metabolites isolated from marine invertebrates collected in Papua New Guinea,[7] it was found that crude extracts of the sponge *Theonella swinhoei* Gray[8] inhibited protein phosphatase-1 even at extremely high dilution. Enzyme assay-guided fractionation[9a] of the crude extracts led to the isolation of motuporin (**1**), a novel cyclic pentapeptide. Motuporin (**1**) inhibits protein phosphatase-1 in a standard phosphorylase phosphatase assay at a concentration of <1 nmolar, making it one of the most potent PP1 inhibitors known,[9] and it also displays considerable *in vitro* cytotoxicity against murine leukemia (P388: IC_{50} 6μg/mL), human lung (A549: IC_{50} 2.4μg/mL), ovarian (HEY: IC_{50} 2.8μg/mL), colon (LoVo: IC_{50} 2.3μg/mL), breast (MCF7: IC_{50} 12.4μg/mL) and brain (U373MG: IC_{50} 2.4μg/mL) cancer cell lines.

Specimens of *T. swinhoei* were collected by hand using SCUBA on nearshore reefs off Motupore Island, Papua New Guinea. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. Thawed sponge (≈250g dry wt.) was extracted repeatedly with methanol (3 x 500mL) and the combined extracts were concentrated in vacuo before being partitioned between hexanes and water. The PP1 inhibitory aqueous phase was next partitioned between $CHCl_3$ and methanol/water (4:7) to give an inhibitory aqueous

phase that was chromatographed on Sephadex LH20 (eluent: methanol) and preparative reversed phase TLC (Whatman RP $KC_{18}F$: methanol/H_2O 6:4) to give pure motuporin (**1**) as an optically active ($[\alpha]_D$ -83.8°) clear glass (≈ 54 mg).[10]

Motuporin (**1**) showed parent ions at m/z 812 (±2) ($C_{40}H_{55}N_5O_{10}Na_2$) and 790 (±2) ($C_{40}H_{56}N_5O_{10}Na$) Da in the LRFABMS. Doping the matrix with potassium chloride shifted the FABMS peaks to m/z 845 (±2) ($C_{40}H_{55}N_5O_{10}K_2$) and 806 (±2) ($C_{40}H_{56}N_5O_{10}K$) in agreement with the presence of one or two metal atoms in the parent ions. Dimethylmotuporin (**2**), prepared by treating motuporin (**1**) with MeI/K_2CO_3 in DMF at rt overnight, gave a parent ion in the EIHRMS at m/z 795.4421 appropriate for a molecular formula of $C_{42}H_{61}N_5O_{10}$ (ΔM +0.3mmu). The peptidic nature of motuporin was indicated by its ^1H and ^{13}C nmr data (Table 1). COSY, HMQC, HMBC and nOe-difference experiments readily identified valine, glutamic acid, β-methylaspartic acid and N-methyl-(Z)-dehydrobutyrine residues in **1** (Table 1).



1 R=H

2 R=Me

The remaining portion of motuporin ($C_{20}H_{27}NO_2$) was shown by analysis of both ^{13}C and ^1H nmr data for **1** and its dimethyl ester **2** (Table 1) to belong to the β-amino acid Adda, that had previously been reported from the blue-green algal toxins microcystin-LR and nodularin.[11] The chemical shifts, coupling patterns and nOe behaviour of the resonances assigned to the Adda residue in motuporin (**1**) were essentially identical to those previously reported for the microcystins[12] and nodularin[11] in agreement with identical constitutions and relative stereochemistries for all known Adda residues. Motuporin's molecular formula ($C_{40}H_{57}N_5O_{10}$) required fifteen sites of unsaturation. Fourteen of these could be accounted for by functionality in the identified amino acid residues and the final site was attributed to a macrocyclic pentapeptide ring.

HMBC and nOe data collected on the dimethyl ester **2** provided information on the amino acid sequence in motuporin. Corrrelations between the methyl ester protons (δ 3.73 and 3.88) and the C1 carboxyl carbons (δ 172.6 and 172.7: Table 1) of the glutamic acid and β-methylaspartic acid residues established the γ-Glu and β-MeAsp peptide linkages. Irradiation of the Glu NH resonance (39NH: δ 6.66) induced nOes in H2 (δ 2.76) and H39 (δ 4.37) demonstrating a peptide link between the Adda carboxyl and the Glu amino group. HMBC correlations from the N-methyl protons (Me35: δ 3.09) of the NMeΔBut residue and a γ-methylene proton (H37': δ2.39) of the Glu residue to the δ-carboxyl group (C36: δ 172.2) of the Glu residue connected Glu to NMeΔBut. HMBC correlations from the NMeΔBut olefinic proton (H33: δ 7.02) and the β-methylaspartic acid NH proton (28NH: δ 8.13) to the NMeΔBut carboxyl carbon (C31: δ164.2) linked NMeΔBut to β-MeAsp and a

Table1	Motuporin (1) (MeOH-d_4)				Dimethylmotuporin (2) ($CDCl_3$)			
Carbon#	^{1}H 500MHz[a]	nOe[b]	^{13}C[c]	HMBC[d]	^{1}H 500MHz[a]	nOe[b]	^{13}C[c]	HMBC[d]
ADDA								
1			176.4	H:17			174.9	39NH
2	3.02,dd(10.5,7)		44.8	H:17	2.76,m		43.8	
3	4.61,dd(10.5,9)		56.0	H:2,5,17	3.87-3.90		59.1	H:5,17
4	5.61,dd(15.5,9)		127.3		5.86,dd(15.5,9)		124.1	
5	6.22,d(15.5)		138.4	H:18	6.11,d(15.5)		138.4	H:7
6			134.2	H:8,18			132.6	H:4,18
7	5.39,d(9.5)		136.4	H:8,18,19	5.32,d(10)		135.9	H:5,8,18,19
8	2.57,m		37.7	H:19	2.55,m		36.7	
9	3.21-3.25		88.5	H:8,10,10',19,20	3.17,m		86.9	H:10,19,20
10	2.65,dd(14,7)		39.0	H:8,12,16	2.66,dd(14,7.5)		38.3	H:12,16
10'	2.81,dd(14,4.5)				2.76,m			
11			140.6	H:10,10',13,15			139.3	H:10,10',13,15
12	7.13-7.20,m		130.5	H:10,10',14	7.16,m		129.4	H:10,10',14,16
13	7.23,m		129.2	H:12,15	7.23,m		128.2	H:15
14	7.13-7.20,m		127.0	H:12,16	7.16,m		126.0	H:12,16
15	7.23,m		129.2	H:13,16	7.23,m		128.2	H13
16	7.13-7.20,m		130.5	H:10,10',14	7.16,m		129.4	H:10,10',14,12
17	1.02,d(7)		16.4[e]		0.99,d(6.5)		14.9	
18	1.62,s	H4,H8	13.0				12.7	H:5,7
19	0.99,d(6.5)		16.5[e]	H:8	0.99,d(6.5)		16.2	H:8
20	3.23,s		58.7		3.21,s		58.6	H:9
3NH					6.89,bd(6)	H22		
VAL								
21			171.8	H:22			170.2	H:22
22	4.40,bd(3.5)		57.7	H:24,25	4.22,dd(9.3,5.7)	H23	57.9	H:25
23	2.49,m		30.1	H:24,25	2.04,m		30.0	H:24,25
24	0.76,d(6.5)[e]		16.5[e]	H:25	0.82,d(7)[f]		17.8[e]	H:22,25
25	0.84,d(7)[e]		19.8	H:24	0.87,d(6.7)[f]	H23	19.4[e]	H:24
22NH					6.06,bd(9.5)			
βMeASP								
26			177.9	H:28,30			173.9	H:28,30
27	3.21-3.25,m		40.8	H:30	2.86,m		40.4	H:28,30
28	4.20,bd(3)		60.4	H:30	4.40,bd(6.7)		56.2	H:30
29			177.1	H:28			172.7[f]	H:28,41
30	1.20,d(7)	H28	16.6[e]		1.30,d(6)	H28,28NH	15.8	
28NH					8.13,bd(6.7)	H35		
NMeΔBUT								
31			165.7	H:33			164.2	H:33,28NH
32			137.9	H:34,35			135.4	H:34
33	6.94,q(7.5)		136.6		7.02,q(7)	H34	136.4	H:34
34	1.74,d(7.5)		13.2	H:33	1.72,d(7)		13.2	H:33
35	3.07,s	H34	35.0		3.09,s		34.6	
GLU								
36			175.7	H:37,37'			172.2	H:35,37'
37	2.04,ddd(16.4,12.2,4.5)		29.7	H:38'	2.04,m		29.2	H:38',39
37'	2.73,ddd(16.4,12.8,4.0)				2.39,m			
38	1.81,m		28.8	H:37,37'	2.05,m		26.0	H:37,37',39
38'	2.27,m				2.55,m			
39	4.45,bt(4.3)		54.4		4.37,td(7.5,3)		52.9	
40			178.3				172.6[f]	H:39,42,39NH
39NH					6.66,bd(7)	H2,H39		
OMe41					3.73,s[g]		52.5[g]	
OMe42					3.88,s[g]		53.4[g]	

[a]Assignments based on double resonance, COSY60 and DQFCOSY. [b]Proton in C# column irradiated. [c]Assignments based on HMQC and HMBC correlations. [d] Protons correlated to carbon resonances in ^{13}C column. [e,f,g] Interchangeable.

nOe from the Adda NH (3NH: δ6.89) to the Val α–proton (H22: 4.22) linked the Adda amino group to the Val carboxyl. The final peptide linkage required to complete the macrocycle had to be between the Val amino group and the γ-carboxyl of the β-MeAsp residue.

The configurations of the amino acids in motuporin (1) were determined by hydrolysis (6N HCl: reflux 12h) and derivatization with Marfey's reagent followed by HPLC analysis.[13] D-Glutamic acid and L-valine were directly identified by this method and D-erythro-β-methyl aspartic acid was identified by comparing the derivatized hydrolysis products of motuporin (1) to the derivatized hydrolysis products of authentic microcystin-LR.[14] As indicated above, nmr analysis showed that the Adda residue in 1 and in microcystin-LR had the same relative configurations. Ozonolysis of motuporin with oxidative workup (H_2O_2), followed by hydrolysis (6N HCl) and derivatization with Marfey's reagent, liberated a second equivalent of derivatized D-erythro-β-methyl aspartic acid from the Adda residue. A similar result was observed when authentic microcystin-LR was ozonized (H_2O_2 workup) and hydrolyzed (6N HCl). Therefore, the Adda residue in motuporin has the 2S,3S,8S,9S configuration found in microcystin-LR and nodularin and the complete structure of motuporin is as shown in **1**.

Motuporin (**1**) is only the second cyclic pentapeptide in this class and it is the first example that has been isolated from a marine source. It differs from the fresh-water blue-green algal toxin nodularin[11b] by the replacement of a polar arginine residue with a nonpolar valine residue. Motuporin is a potent PP1 inhibitor and cytotoxin which demonstrates that the arginine residue is not essential for biological activity in this class of peptides. Inhibitors are vital tools in the study of the role of protein phosphatases in cell biology. Motuporin should help contribute to our understanding of these enzymes, which regulate important hormone and growth factor intracellular signalling pathways.[9] The structural similarity between motuporin and nodularin provides strong evidence that motuporin is being produced by a blue-green alga.

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