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Solomonamides A and B, New Anti-inflammatory Peptides from *Theonella swinhoei*

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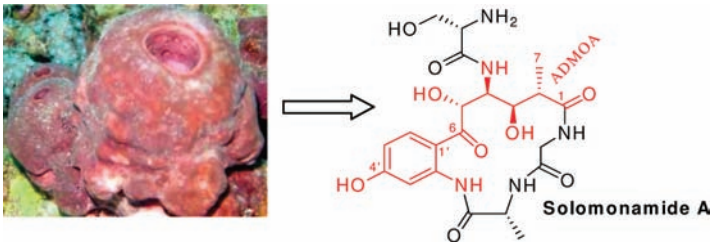
ABSTRACT



Two unprecedented cyclic peptides, solomonamides A and B, were isolated from the marine sponge *Theonella swinhoei*. The structures were elucidated on the basis of comprehensive 1D and 2D NMR analysis and high-resolution mass spectrometry. A combined approach, involving Marfey's method, QM *J* based analysis, and DFT *J*/^{13}C calculations, was used for establishing the absolute configuration of the entire molecule. Solomonamide A showed in vivo anti-inflammatory activity.

The marine sponge *Theonella swinhoei* (Lithistida, Theonellidae) represents a prolific source of innovative and bioactive metabolites. Since the discovery of swinholide,[1] a complex dimeric polyketide lactone isolated and characterized 30 years ago, the impressive variety of unusual new chemical entities from this species continue to fascinate the scientific community for the unusual structures and the powerful biological activities. Among the nine biosynthetic classes of metabolites found from worldwide collections of *Theonella swinhoei*,[2] surely the peptides represent the most significant group. Recently we reported the isolation of two potent anti-inflammatory octacyclopeptides, perthamides C and D from a Solomon collection of *Theonella swinhoei*.[3] Pursuing the chemical investigation of the polar extracts of the sponge, we isolated two minor peptide derivatives unrelated to peptides previously found from marine sources, which we named solomonamides A and B. In this paper we describe the isolation, the structure elucidation including the stereochemical characterization, and the biological activity of the new peptides.

Solomonamide A (**1**)[4] was isolated as a minor (6.2 mg) peptide component of sponge polar extracts. A molecular formula of $C_{21}H_{29}N_5O_9$ was established by HR ESIMS mass spectrometry. Analysis of NMR spectra revealed four exchangeable amide NH protons between δ_H 11.5 and 7.49 and four acyl carbonyls (δ_C 167.5, 169.2, 170.7,

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(4) White amorphous solid; $[\alpha]^{25}_D$ +2.3 (*c* 0.17, MeOH).

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and 173.6). The presence of three conventional amino acid residues, alanine, glycine, and serine, was easily deduced by interpretation of 2D NMR data obtained by $^1H-^1H$ COSY, HSQC, and HMBC experiments. A signal at δ 8.10, integrating for two protons, was assigned as the α-amino group of the serine residue, indicating that the above residue is the N-terminus.

Table 1. NMR Spectroscopic Data for Solomonamide A (**1**) in DMSO-d_6

residue		δ_C	δ_H	HMBC
Gly	1	169.2		
	2a	42.6	3.90 dd (15.3, 6.6)	1, 1_{ADMOA}
	2b		3.76 dd (15.3, 4.7)	1, 1_{ADMOA}
	NH		7.49 br t (5.5)	2, 1_{ADMOA}
Ala	1	170.7		
	2	49.1	4.41 quint (7.6)	1, 3, 1_{Gly}
	3	15.4	1.31 d (7.0)	1, 2
	NH		8.77 d (7.9)	2, 3, 1_{Gly}
Ser	1	167.5		
	2	54.2	4.02 m	1, 3
	3a	60.5	3.81 dd (11.4, 4.6)	1
	3b		3.68 dd (11.4, 6.9)	1, 2
	NH_2		8.10 br d (4.3)	1, 2, 3
	OH		5.39 br s	
ADMOA	1	173.6		
	2	44.3	2.34 dq (9.7, 7.1)	1, 7, 3, 4
	3	70.3	3.15 ovl[a]	1, 2
	4	53.3	4.54 t (9.6)	2, 3, 6, 1_{Ser}
	5	70.4	4.75 d (9.6)	4
	6	201.2		
	7	13.6	0.98 d (7.1)	1, 2, 3
	OH-3		5.26 br s	
	OH-5		5.53 br s	
	NH-4		7.89 d (9.6)	1_{Ser}, 3
	1′	115.3		
	2′	141.8		
	3′	106.0	8.02 d (2.3)	1′, 2′, 4′, 5′
	4′	162.8		
	5′	109.6	6.57 dd (8.7, 2.3)	1′, 3′, 4′
	6′	133.4	7.86 d (8.7)	6_{ADMOA}, 2′, 4′
	OH		10.7 s	3′, 4′, 5′
	NH-2′		11.5 s	1′, 3′, 1_{Ala}

[a] Ovl: overlapped with residual H_2O in DMSO-d_6.

The structure of unprecedented ADMOA residue was deduced as follows. The $CH_3(7)-CH(2)-CH(3)-OH$ and $CH(4)NH-CH(5)-OH$ spin systems were disclosed from COSY and HSQC data. Even if no COSY correlation was observed between H3 and H4, the connection between C3 and C4 was deduced by HMBC data (Table 1 and Figure 1). The diagnostic HMBC correlations H2, H3, and H_3-7 to the carbon resonance at δ_C 173.6 allowed the placement of an acyl carbonyl at C1. Moreover a ketone carbonyl group was placed at C6 of the spin system on the basis of ^3J HMBC correlation between $CH(4)-NH$ (δ_H 4.54) with a carbonyl group at δ 201.2 (C6). In addition, the ^1H NMR together with COSY, HSQC, and HMBC data showed the presence of a 1,2,4 trisubstituted phenyl ring (Table 1 and Figure 1). The ketonic carbonyl at C6 of the above-mentioned spin system was placed at C1′ of the phenyl ring on the basis of the HMBC cross peaks between the C6′ aromatic proton at δ 7.86 with the signal at δ 201.2. One phenolic hydroxy and one aromatic amido group deduced by mass and ^{13}C NMR data were also evidenced by two downfield signals, observed in the ^1H NMR spectrum at δ 10.7 and 11.5, respectively. The relative regiochemistry of oxygenated and nitrogen functionalities on the phenyl ring was secured by HMBC correlations shown in Figure 1. Therefore the new unit in solomonamide A was defined as 4-amino(2′-amino-4′-hydroxyphenyl)-3,5-dihydroxy-2-methyl-6-oxohexanoic acid (ADMOA).

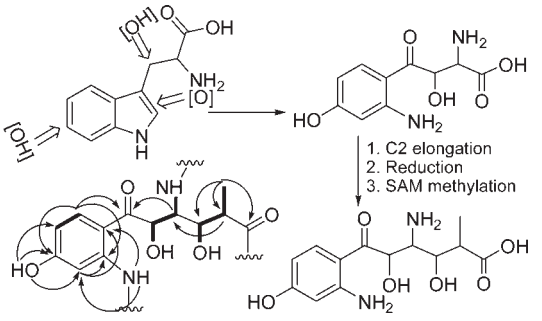

Figure 1. Plausible biogenesis, COSY/TOCSY connectivities (bold bonds), and key HMBC correlations (arrows) for ADMOA unit in solomonamide A (**1**).

A plausible biogenetic origin of this unit could be drawn: 5-hydroxytryptophan could undergo two well-known processes of tryptophan catabolism, i.e., β-hydroxylation[5] and oxidative scission of the indole ring,[6] to afford a dihydroxykynurenine unit. Modification by a PKS extension, reduction of the ketone to an alcohol, and methylation of the acetate C-2 carbon[7] (Figure 1) would eventually afford the ADMOA residue.

The amino acid sequence of **1** was established from the interpretation of HMBC data (Table 1), where long-range correlations between protons on α-carbon and on α-amido groups and carbonyl carbons belonging to adjacent amino acids provided the partial sequence ADMOA-Gly-Ala. Cyclization between the C-terminal alanine and the ADMOA 2′-amino group was inferred from HMBC correlation between the 2′-amino proton of ADMOA (δ_H 11.5) and the carbonyl resonance at δ_C 170.7 (C1 Ala). The linkage of γ-CHNH of ADMOA residue to the acyl group of the N-terminal serine residue was determined by long-range correlations between protons at δ_H 7.89 (NH-4) and δ_H 4.54 (C4) to C1 Ser (δ_C 167.5).

Several inter-residue ROESY correlations confirmed the proposed sequence (Figure 2). Definitive confirmation of the sample structure was derived from ESI MS/MS

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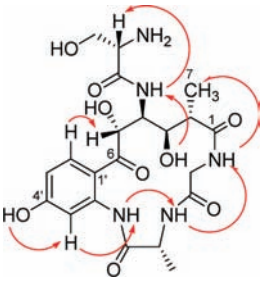

Figure 2. Key ROESY correlations for solomonamide A (**1**).

analysis. In addition to the pseudomolecular ion at *m*/*z* 496.2 $[M + H]^+$, the ESI Q-TOF MS/MS spectrum provided some fragment ion peaks, in agreement with the proposed sequence.

The major peak at *m*/*z* 374 corresponds to the simultaneous dehydratation at C2−C3 and eliminative loss of the terminal serine residue. Further MS3 fragmentation of the daughter ion at *m*/*z* 374 gave C-terminus ion fragments at *m*/*z* 303 $[374 - Ala]^+$ and *m*/*z* 246 $[374 - Ala - Gly]^+$.

The absolute configuration of Ala and Ser residues was determined by complete acid hydrolysis of solomonamide A and Marfey's analysis.[8] The acid hydrolysate was derivatized with L-FDAA, and then LC-MS comparison of the derivatives from parent peptide with the FDAA derivatives of appropriate standards established the presence of D-Ala and L-Ser.

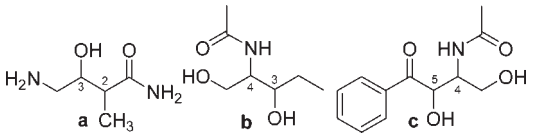

Figure 3. Molecular fragments representing the three C2 reduced systems of ADMOA. Numbers are referred to the unit in **1**.

The determination of the configuration of ADMOA in solomonamide A, consisting of four stereocenters, was initially approached by applying the Quantum Mechanical (QM) *J* method.[9] Following this method, we designed three C2 fragments (**a**−**c**, Figure 3), containing a single pair of stereocenters. For each fragment, three (one *anti* and two *gauche*) staggered arrangements were considered for each of the two *erythro* and *threo* stereochemical series.

Subsequently each arrangement was optimized at the MPW1PW91 level of theory by using the 6-31G(d) basis set and then the calculation of *J* couplings on the optimized geometries was executed by using the same functional and the 6-31G(d,p) basis set. For both calculation steps, the IEF-PCM solvent continuum model (DMSO solvent) was used. The results of the calculation were compared with the experimental data (Table S3, Supporting Information).

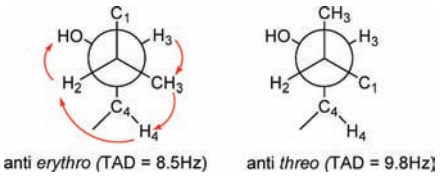

Figure 4. C2−C3 anti rotamers with diagnostic ROE effects (red arrows).

Despite the C2−C3 fragment the difference between the TAD values (Total Absolute Deviations, Table S3, Supporting Information) for the anti *erythro* configuration vs the anti *threo* configuration was only 1.3 Hz, and the presence of diagnostic ROE (Figure 4) allowed us to unambiguously established an *erythro* arrangement. On the other hand, for C3−C4 and C4−C5 fragments, small differences in the TAD values (Table S3, Supporting Information) did not allow confident assignments of their relative configurations. To solve this issue and to propose the absolute configuration of ADMOA residue in **1**, we proceeded with the QM calculation of diagnostic *J* coupling constants and the ^{13}C chemical shift values for the whole molecule.

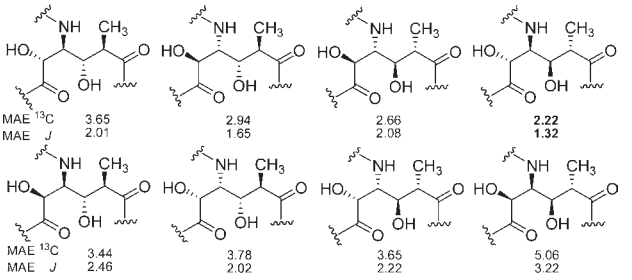

Figure 5. Mean absolute error (MAE) found for ^{13}C NMR chemical shifts and *J* values of the eight diastereoisomers: MAE $^{13}C = \sum[|(\delta_{exp} - \delta_{calc})|]/n$; MAE $J = \sum[|(J_{exp} - J_{calc})|]/n$.

Eight diastereoisomers (Figure 5) were built, varying the configuration at four stereocenters of the ADMOA residue, fixing the *erythro* arrangement for C2−C3 and the absolute configuration of Ala and Ser, previously established through Marfey's method. On the eight compounds, we followed a protocol successfully applied in previous stereostructural determination:[10,11] (a) conformational search and geometry optimization of all the significant conformers of each stereoisomer, obtained by molecular mechanics and dynamics (Supporting Information) considering the available

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experimental restraints; (b) final geometry optimization of all the species at the DFT level, by using the MPW1PW91 functional and the 6-31G(d) basis set; (c) GIAO (gauge including atomic orbital) ^{13}C NMR and *J* coupling constant calculations of so-obtained structures using the same functional and the 6-31G(d,p) basis set (see the Supporting Information); and (d) comparison of *J* values and ^{13}C chemical shifts calculated for each stereoisomer with those experimentally measured for **1**.

MAE (Mean Absolute Error) parameters were obtained for each of eight diastereoisomers. Through evaluation of the MAE values, the GIAO calculated ^{13}C NMR chemical shifts of ^{13}C sp^3 atoms and the *J* coupling constants indicated the best fit with the experimental data for stereoisomer 2*S*, 3*R*, 4*S*, 5*R* (Tables S4−S7 in the Supporting Information, and Figure 5).

In conclusion the application of the QM method allowed us to propose for the ADMOA residue the absolute configuration 2*S*, 3*R*, 4*S*, 5*R*, which was further supported by a retrospective analysis of diagnostic ROESY cross-peaks (Figure S12 in the Supporting Information).

The HR ESIMS of solomonamide B (**2**)[12] showed a major ion peak at *m*/*z* 480.2067 $[M + H]^+$ ($C_{21}H_{30}N_5O_8$, calcd for $C_{21}H_{30}N_5O_8$, 480.2094), 16 mass units lower than **1**. Comparison of 2D NMR data (Table S2 in Supporting Information) of solomonamide B (**2**) with those of **1** clearly evidenced a close analogy between two compounds and a perturbation in the ADMOA residue. The signal assigned to H-4 hydroxymethine of the above residue in **1** was replaced by signals relative to a diasterotopic methylene (δ_H 3.34 and 2.87; δ_C 41.2). 2D NMR analysis clearly evidenced that solomonamide B differs from solomonamide A for the presence of 4-amino-6-(2′-amino-4′-hydroxyphenyl)-3-hydroxy-2-methyl-6-oxohexanoic acid residue (AHMOA) instead of ADMOA residue (Figure 6).

The presence of D-Ala and L-Ser was established by the Marfey method as described above. The complete matching of the $^3J_{H,H}$ coupling constants pattern between the nuclei of AHMOA residue with the corresponding ones in ADMOA suggested a common configuration of C2−C4 stereogenic centers (Figure 6).

(12) White amorphous solid; $[\alpha]^{25}_D$ +4.8 (*c* 0.28, MeOH).

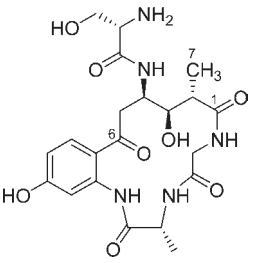

Figure 6. Solomonamide B (**2**).

Solomonamide A (**1**) displayed a dose-dependent anti-inflammatory activity causing about 60% reduction (see the Supporting Information) of edema in mice at the dose of 100 μg/kg (ip). The scarcity of the material hampered the evaluation of anti-inflammatory activity for **2**.

In conclusion, solomonamide A is representative of a new class of anti-inflammatory compounds. The presence of this suite of compounds in a single collection of a *Theonella swinhoei* further emphasizes the chemical diversity present in the lithistid sponges.

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Supporting Information Available. Experimental procedures, spectroscopic data for compounds **1** and **2**, QM *J* based analysis and DFT $J/^{13}C$ calculated values for **1** and pharmacological assays. This material is available free of charge via the Internet at http://pubs.acs.org.