Theopalauamide, a Bicyclic Glycopeptide from Filamentous Bacterial Symbionts of the Lithistid Sponge *Theonella swinhoei* from Palau and Mozambique

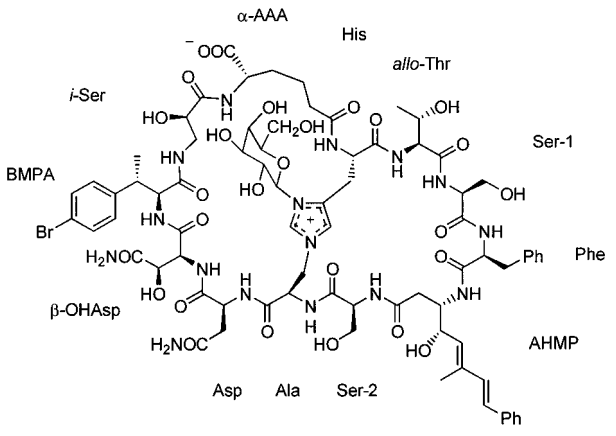
Eric W. Schmidt, Carole A. Bewley, and D. John Faulkner*

Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0212

Received October 6, 1997

Theopalauamide (**1**) is the major bicyclic peptide from the symbiotic filamentous eubacteria that are found in the interior of *Theonella swinhoei* from Palau. It was also isolated from *T. swinhoei* from Mozambique. The structure of theopalauamide was determined by analysis of spectroscopic data, and its stereochemistry was determined by chemical degradation and analysis of the products by chiral GC-MS. The minor peptide, isotheopalauamide (**2**), was shown to be a stable conformational isomer that was formed by TFA-catalyzed equilibration during the isolation procedure.

The metabolites of lithistid sponges, which include the genera *Theonella*, *Discodermia*, *Aciculites*, *Microscleroderma*, and *Callipelta*, are among the most diverse found in any order of sponges and have often been attributed to symbiotic microorganisms.[1] Our studies of the cellular location of the metabolites of *Theonella swinhoei* from Palau led to the discovery that the cytotoxin swinholide A was associated with a mixture of unicellular bacteria and that the major peptide, which was referred to as P951, was located in filamentous eubacteria that are found only in the interior of the sponge.[2] At the time that the localization studies were performed, the structure of P951, now called theopalauamide (**1**), was tentatively assigned but the presence of a minor peptide complicated the assignment. We now present the structural elucidation of theopalauamide (**1**) and define its relationship with the minor peptide isotheopalauamide (**2**).[3]



1 and **2**

A lyophilized specimen of *T. swinhoei* from Palau was extracted sequentially with hexanes, dichloromethane, ethyl acetate, and 1:1 acetonitrile/water, as described previously.[4] The aqueous acetonitrile extract was partially evaporated to obtain a peptide fraction as a white solid. Initially, the peptides were purified by chromatography on a reversed phase C_{18} silica Sep Pak column using an acetonitrile/0.05% aqueous TFA gradient followed by reversed-phase HPLC again using acetonitrile/0.05% aqueous TFA as eluant to obtain both theopalauamide (**1**) and isotheopalauamide (**2**). We then realized that theopalauamide (**1**) was undergoing isomerization during the purification process. We suspected that the isomerization might be acid-catalyzed but elimination of TFA from the HPLC eluant gave broader peaks and rendered preparative-scale separation impractical. A compromise separation scheme used on the Mozambique specimen employed an aqueous acetonitrile gradient as eluant for reversed-phase Sep Pak separations and 38% acetonitrile in 0.01% aqueous TFA for the final reversed-phase HPLC separation to obtain almost pure theopalauamide (**1**), in which only a trace of **2** could be detected by ^{1}H NMR spectroscopy (see below). The same conditions were used on a 1:1 mixture of peptides from the Palau specimen of *T. swinhoei* to separate pure samples of theopalauamide (**1**), which was identical to the material from the Mozambique sponge, and isotheopalauamide (**2**).[5]

Theopalauamide (**1**) was obtained as a white powder that decomposed before melting. The molecular formula, $C_{76}H_{99}BrN_{16}O_{27}$, was deduced from high-resolution mass measurement and analysis of the ^{13}C NMR spectrum, which contained 76 carbon signals. The molecular formula differs from that of theonegramide (**3**, $C_{75}H_{97}BrN_{16}O_{26}$) by addition of "$CH_2O$", and that difference was compatible with the replacement of the pentose sugar (D-arabinose) in **3** by a hexose in **1**. The sugar was identified as galactose on the basis of ^{1}H and ^{13}C NMR data and by analysis of key ^{1}H NMR coupling constants (see Table

(1) (a) Bewley, C. A.; Faulkner, D. J. *Angew. Chem., Int. Edit. Engl.* Submitted. (b) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259−302 and previous reports in this series.

(2) Bewley, C. A.; Holland, N. D.; Faulkner, D. J. *Experientia* **1996**, *52*, 716−722.

(3) The name isotheopalauamide is used because compound **2** is a stable conformational isomer of theopalauamide (**1**) with different physical and spectral properties. There does not appear to be a special prefix to describe this situation.

(4) Bewley, C. A.; Faulkner, D. J. *J. Org. Chem.* **1994**, *59*, 4849−4852.

(5) We eventually found that isomerization occurred during removal of the solvent after HPLC separation and that isomerization could be suppressed by addition of ammonia to neutralize the TFA.

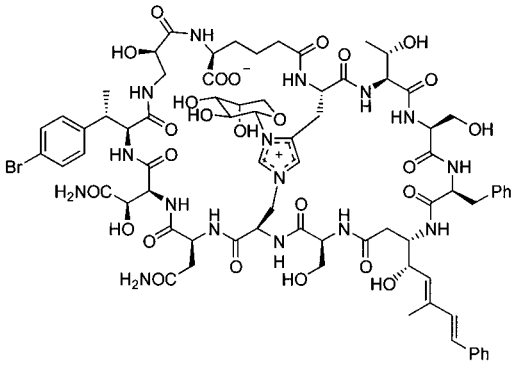
S0022-3263(97)01845-8 CCC: $15.00
Published on Web 01/30/1998

Table 1. ^{1}H (DMF-d_7) and ^{13}C (DMF-d_7) NMR Data for Theopalauamide (1).

amino acid	C	δ_C	δ_H	mult, J (Hz)	amino acid	C	δ_C	δ_H	mult J (Hz)
allo-Thr	1	173.4				4	171.3		
	2	59.1	4.58	t, 10		NH		8.30	d
	3	69.1	3.86	m		NH_2		6.97	br s
	4	21.7	1.16	d, 5.5				7.70	br s
	OH		6.08						
	NH		7.87	d, 8	β-OHAsp	1	171.3		
						2	54.6	5.73	br t, 5
Ser−1	1	170.1				3	72.2	4.21	br
	2	57.8	4.80	m		4	170.8		
	3	62.3	3.70	m		NH		8.69	d, 10
			3.84	m		NH_2		7.46	br s (2H)
	NH		8.97	d, 10		OH		4.79	
Phe	1	172.4			BMPA	1	174.2		
	2	54.9	4.88	q, 8		2	59.1	4.76	m
	3	39.8	2.77	m		3	38.7	3.75	m
			2.97	dd, 13.5, 7		4	17.9	1.19	d, 5
	4	137.8				5	141.6		
	5,9	128.9	7.29	s		6,10	129.3	7.37	d, 8
	6,8	130.0	7.29	d, 8		7,9	131.6	7.14	d, 8
	7	127.2	7.23	t, 8		8	120.6		
	NH		8.28	d, 8		NH		8.06	d
AHMP	1	172.7			*i*-Ser	1	171.9		
	2	37.9	2.34	br d, 13.5		2	70.4	4.29	br
			2.70	m		3	44.3	3.12	br d, 10
	3	53.7	4.41	m				4.00	m
	4	68.9	4.48	m		NH		7.44	d, 7.5
	5	134.1	5.43	d, 8.5					
	6	135.7			α-AAA	1	173.7		
	7	134.1	6.83	d, 15.5		2	35.1	1.82	m
	8	128.2	6.60	d, 15.5				2.27	m
	9	138.3				3	22.2	1.00	m
	10,14	127.0	7.54	d, 7				1.40	m
	11,13	131.6	7.38	t, 8		4	31.8	1.66	m
	12	128.0	7.26	t, 8				1.82	m
	15	13.0	1.79	s		5	51.9	4.36	m
	NH		8.49	d, 9.5		6	174.1		
						NH		7.68	d, 8.5
Ser−2	1	171.9							
	2	57.5	4.00	m	His	1	170.1		
	3	61.5	3.71	m		2	54.9	5.05	m
			3.94	m		3	27.2	3.05	dd, 16, 3
	NH		8.18	br s				3.60	t, 16
						4	131.7		
Ala	1	169.8				5	124.8	7.47	br s
	2	51.4	5.26	br d, 10		7	137.5	9.49	br s
	3	50.7	4.43	m		NH		8.92	d, 8
			5.10	br d, 9					
	NH		8.61	d, 10.5	Gal	1	90.1	5.47	d, 8.5
						2	70.9	3.76	m
Asp	1	172.1				3	74.6	3.53	dd, 11.5, 2
	2	52.6	4.70	m		4	71.1	4.01	m
	3	37.1	2.49	br d, 13.5		5	78.9	3.93	m
						6	63.5	3.98	m

1). The coupling constant of the anomeric proton at δ 5.47 (d, 1 H, J = 8.5 Hz) indicated that H-1 and H-2 were both axial and those of the H-3 proton at 3.53 (dd, 1 H, J = 11.5, 2 Hz) required H-3 to be axial and H-4 to be equatorial. The H-4, H-5, and H-6 signals were overlapping, but comparison of the corresponding ^{13}C chemical shifts with literature values[6] strongly suggested the galactose stereochemistry. Mild acid hydrolysis of theopalauamide (**1**) produced D-galactose, which was identified by derivatization and comparison with authentic samples of both D- and L-galactose using GC-MS with a chiral support.

Having identified the sugar residue, it was essential to confirm that the bicyclic peptide portion of theopalauamide (**1**) was identical to that of theonegramide (**3**).



3

This was most easily accomplished by performing a complete structural elucidation. The DQCOSY and TOCSY spectra were used to determine the identity of most of the amino acids. The HMQC and HMBC experi-

(6) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972; p 461.

Table 2. Comparison of NMR Data for the Pheynylalanine and AHMP Residues of Theopalauamide (1) and Isotheopalauamide (2)

		1			2		
amino acid	C	δ_C	δ_H	mult, *J* (Hz)	δ_C	δ_H	mult, *J* (Hz)
Ser−1	1	170.1			170.4		
	2	57.8	4.80	m	57.9	4.70	m
Phe	1	172.4			172.2		
	2	54.9	4.88	q, 8	55.1	4.73	m
	3	39.8	2.77	m	39.7	2.70	m
			2.97	dd, 13.5, 7		2.81	m
	4	137.8			138.3		
	5,9	128.9	7.29	s	129.9	7.23	s
	6,8	130.0	7.29	d, 8	128.8	7.24	m
	7	127.2	7.23	t, 8	128.0	7.25	m
	NH		8.28	d, 8		8.26	d, 9.5
AHMP	1	172.7			173.1		
	2	37.9	2.34	br d, 13.5	37.8	2.50	m
			2.70	m		2.84	m
	3	53.7	4.41	m	53.8	4.43	m
	4	68.9	4.48	m	70.4	4.42	m
	5	134.1	5.43	d, 8.5	134.4	5.64	d, 8.5
	6	135.7			136.4		
	7	134.1	6.83	d, 15.5	134.0	6.92	d, 15.5
	8	128.2	6.60	d, 15.5	128.6	6.65	d, 15.5
	9	138.3			138.3		
	10,14	127.0	7.54	d, 7	127.0	7.54	m
	11,13	131.6	7.38	t, 8	131.6	7.38	m
	12	128.0	7.26	t, 8	128.2	7.26	m
	15	13.0	1.79	s	13.3	1.92	s
	NH		8.49	d, 9.5		8.53	d, 12
Ser−2	2	57.5	4.00	m	57.7	3.95	m
Hist	7	137.5	9.49	br s	137.8	9.32	br s

ments confirmed these assignments and allowed identification of the aromatic amino acids. The amino acid sequence was determined from the HMBC and ROESY (mixing time = 300 ms) data and was identical to that of theonegramide. The absolute configuration of each amino acid in theopalauamide was shown to be identical to that in theonegramide by employing the same series of chemical degradations, followed by chiral GC-MS analysis of the fragments, that had been used previously.[4] These experiments established that theopalauamide (**1**) and theonegramide (**3**) differed only in the identity of the sugar unit.

Isotheopalauamide (**2**) has the same molecular formula as theopalauamide (**2**), and both have identical IR and UV spectra. The differences between the ^{1}H and ^{13}C NMR data for **1** and **2** are mainly confined to signals associated with the phenylalanine and 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid (AHMP) units (Table 2). The most noticeable differences in the ^{1}H NMR spectra were associated with the vinyl methyl signal (δ 1.79 in **1** vs 1.92 in **2**) and the olefinic proton signals on the AHMP residue. In fact, the strength of the δ 1.92 signal could be used to determine the purity of samples of both theopalauamide (**1**) and, interestingly, theonegramide (**3**), which appeared to have isomerized to a lesser extent. Analysis of the DQCOSY, TOCSY, HMQC and HMBC data indicated that the primary structures of **1** and **2** were identical. The GC-MS data for **1** and **2** were identical except that different retention times were observed for the hydrogenation product of AHMP. From these experiments, however, it was not possible to determine whether the differences were due to the stereochemistry of the AHMP residue or to epimers at C-6 caused by hydrogenation occurring from different faces of the diene in peptides **1** and **2**.

Having first ruled out the possibility that **2** was simply the TFA salt of **1**, we initially sought to show that the spectral differences were caused by acid-catalyzed epimerization of the C-4 allylic alcohol in the AHMP residue. To test this hypothesis, a 1:1 mixture of peptides **1** and **2** was hydrogenated and hydrolyzed to obtain a mixture of two lactones arising from the AHMP residue. In theory, four isomers should have been produced but we were not concerned at that point because Matsunaga *et al.*[7] had noted in their elegant studies of theonellamide F that hydrogenation of the AHMP residue appeared to produce predominantly one epimer at the C-6 carbon bearing the secondary methyl group. The lactones were then converted into their *N*-2,4-dinitrophenyl derivatives, which were separated using HPLC to obtain lactones **4** and **5** in a ratio of 1.7:1. The NOESY spectrum of the major (3*S*,4*S*)-lactone **4**, which was identical to that reported by Matsunaga et al.,[7,8] showed a correlation between H-3 and H-4 as expected. However, the ^{1}H NMR and NOESY spectra of lactone **5** were almost identical to those of lactone **4**, particularly with regard to those signals associated with the lactone ring (Figure 1). A careful analysis of the NOESY spectra of lactones **4** and **5** revealed that both compounds had the same relative stereochemistry about the lactone ring. Since the major chemical shift differences were associated with the methyl signals, we concluded that the lactones **4** and **5** were epimers at C-6 that had been generated during the hydrogenation step. After reviewing all of the spectra generated during the reaction sequence described above,

(7) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Walchli, M. *J. Am. Chem. Soc.* **1989**, *111*, 2582−2588.

(8) Matsunaga et al.[7] reported that hydrogenation of theonellamide F followed by hydrolysis of the peptide and 2,4-DNP derivatization gave a 5:1 mixture of epimers, of which the major isomer was identical to lactone **4**. When we hydrogenated theonellamide F and performed the standard hydrolysis and derivatization for GC-MS analysis, we found that the retention time of the minor peak resulting from the AHMP residue was approximately the same as that of the major peak produced by hydrogenation, hydrolysis, and derivatization of isotheopalauamide (**2**).

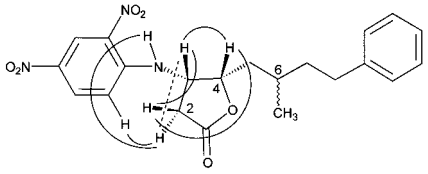


Figure 1. Key NOE correlations for lactones **4** and **5**, which are epimeric at C-6. NOESY spectra (500 MHz) were virtually identical for both compounds. Strong NOE correlations are shown as solid lines and a weak correllation as a dashed line.

we could find no evidence of epimerization at C-4 or at any other chiral center.

In the ROESY spectrum of isotheopalauamide (**2**), there is a correlation between the NH protons of the Phe and AHMP residues that is absent in the ROESY spectrum of **1**. Conversely, the correlation between the NH proton of the AHMP residue and the α-proton of the Phe residue was observed in **1** but not in **2**. The lack of correlations between the α-protons of the Phe and AHMP residues in **2** suggests that the geometry of the amide bond is anti as usual. Thus the only remaining explanation of the difference between the two peptides is that they are different stable conformers of the same compound. Since NMR chemical shift differences in exchangeable proton signals indicate the degree of intramolecular hydrogen bonding,[9] the ^{1}H NMR spectra of compounds **1** and **2** were acquired every 5 °C from 25 to 40 °C. Analysis of chemical shift differences showed that the −NH protons on both the AHMP and Phe residues were more strongly hydrogen bonded in **2** than in **1**, which is consistent with the ROESY data, while most other chemical shift differences were roughly equal. In **1**, the AHMP and Phe −NH proton signals had $\Delta\delta/\Delta T$ (ppb/K) values of 6.5 and 7.1, respectively, while the same protons in **2** had $\Delta\delta/\Delta T$ values 3.1 and 1.5, respectively. The NH−CHα coupling constants for Phe and AHMP were slightly larger in **2** than in **1**, but the differences are not great enough to indicate a major change in the geometry about these bonds. Thus it appears that the major conformational difference between **1** and **2** is a rotation of the bond between C-1 and C-2 in the Phe residue, as shown in Figure 2, with minor adjustments to the geometry of other bonds in the ring. Space-filling models of theopalauamide indicate that such a rotation moves the phenyl ring of Phe and the AHMP side chain further apart in **2** than in **1**, which is consistent with both the differences in NMR data for the two molecules (Table 2) and the hydrogenation data that suggests that one face of the AHMP diene system is sterically hindered in **1** but not in **2**. Having determined that theopalauamide (**1**) and isotheopalauamide (**2**) were simply conformational isomers, we were surprised at their apparent stability for we were unable to find any other conditions to isomerize the pure compounds other than during chromatography in the presence of TFA and subsequent evaporation of the solvents.[10]

Both theopalauamide (**1**) and isotheopalauamide (**2**) inhibited the growth of *Candida albicans*. In the standard paper disk assay, **1** was active at 10 μg/disk while **2** was active at 50 μg/disk.

(9) Morita, H.; Kayashita, T.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1997**, *53*, 1607−1616.

(10) A search of the marine natural product literature failed to provide other examples of the isolation of stable conformers of cyclic peptides.

Experimental Section[11]

Isolation of Theopalauamide (1), Isotheopalauamide (2), Swinholide A, and Theonellasterol. Mixtures of theopalauamide (**1**) and isotheopalauamide (**2**) were obtained from *T. swinhoei* from Palau and from the filamentous bacteria as described previously.[2] The mixture was separated by reversed-phase HPLC on a C_{18} preparative column using 35% acetonitrile in water containing 0.01% TFA to obtain pure samples of theopalauamide (**1**) and isotheopalauamide (**2**).

Theopalauamide (**1**) and swinholide A were obtained from a specimen of *T. swinhoei* from Mozambique (MOZ 95-004) using a slightly modified procedure. The sponge (211 g dry weight) was lyophilized and sequentially extracted with 1:1 hexanes/CH_2Cl_2 (3 × 1 L), EtOAc (3 × 1 L), and 1:1 EtOAc/acetone (3 × 1 L). The sponge was then exhaustively extracted with 1:1 acetonitrile/water until no further peptide was detected in the crude extracts by TLC. The aqueous extracts were dried by rotary evaporation until most CH_3CN was removed and a white precipitate had formed. The suspension was centrifuged, and the supernatant removed. The precipitate was applied directly to the top of a C_{18} Sep Pak (Waters) column and subjected to reversed-phase chromatography using an acetonitrile/water gradient (0−100% CH_3CN in 10% increments). Fractions eluting with 40−60% acetonitrile were enriched with a single peptide, which was purified by reversed-phase HPLC (38% CH_3CN, 0.01% TFA) to obtain theopalauamide (**1**, 55.7 mg, 0.026% dry weight). Fractions eluting with 80% acetonitrile were combined to yield impure swinholide A, which was subsequently repurified by silica flash chromatography (100% EtOAc) to obtain swinholide A (21.3 mg, 0.01% dry weight). Additional swinholide A was present in the EtOAc/acetone extract. The hexane/CH_2Cl_2 extract was partitioned between hexane and methanol (8 mL each). The hexane fraction was evaporated under vacuum to obtain a residue that was purified by flash chromatography on silica gel using a hexane/ethyl acetate gradient (20−100% EtOAc). The 30% EtOAc fraction contained pure theonellasterol (28.2 mg, 0.013% dry weight).[12]

Theopalauamide (1): white powder; $[\alpha]_D = +19°$ ($c = 0.4$, MeOH); UV (MeOH) 203 nm (ε 19,600), 276 (ε 9400), 285 (ε 9900), 304 nm (ε 4500); IR (AgCl) 3300, 2920, 1660, 1540 cm^{-1}; ^{1}H NMR (500 MHz, DMF-d_7) see Table 1; ^{13}C NMR (75 MHz, DMF-d_7) see Table 1; HRFABMS *m/z* 1769.5944 $(M + Na)^+$, calcd for $C_{76}H_{99}{}^{79}BrN_{16}O_{27}Na$, 1769.5947.

Isotheopalauamide (2): $[\alpha]_D = +35°$ ($c = 0.04$, MeOH); UV and IR data were identical to those of **1**; ^{1}H (500 MHz, DMF-d_7) and ^{13}C NMR (75 MHz, DMF-d_7) data were identical, within the limits of error, to those reported for **1** in Table 1 except for the signals listed in Table 2; HRFABMS *m/z* 1747.6233 $(M + H)^+$, calcd for $C_{76}H_{100}{}^{79}BrN_{16}O_{27}$, 1747.6127.

Hydrolysis and Derivatization of Hydrolysates. The glycopeptides **1**, **2**, or **3** (100−500 μg) were dissolved in 6 N HCl (500 μL) and heated to 110 °C in tightly sealed 1 mL conical vials for 15 h. HCl was removed under a stream of nitrogen. The hydrolysates were dissolved in 2-propanol (400 μL), to which was added acetyl chloride (100 μL). The vials were quickly capped, and the solutions were heated to 100 °C for 1 h. Excess reagents were removed under nitrogen, and the residue was redissolved in CH_2Cl_2 (400 μL). Pentafluoropropionic anhydride (400 μL) was added, and the reaction mixture was heated to 100 °C for 15 min. Reagents were removed under nitrogen, and the derivatized hydrolysates were redissolved in EtOAc for GC-MS analysis.

Identification of D-Galactose. Both theopalauamide (**1**, 500 μg) and isotheopalauamide (**2**, 500 μg) were separately dissolved in 4 N HCl (500 μL) and heated to 70 °C for 12 h. The solvent was removed, and the hydrolysates were derivatized as described above to obtain a single peak in the GC-MS that corresponded to D-galactose.

(11) For general experimental procedures, see: Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 779−782.

(12) Kho, E.; Imagawa, D. K.; Rohmer, M.; Kashman, Y.; Djerassi, C. *J. Org. Chem.* **1981**, *46*, 1836−1839.

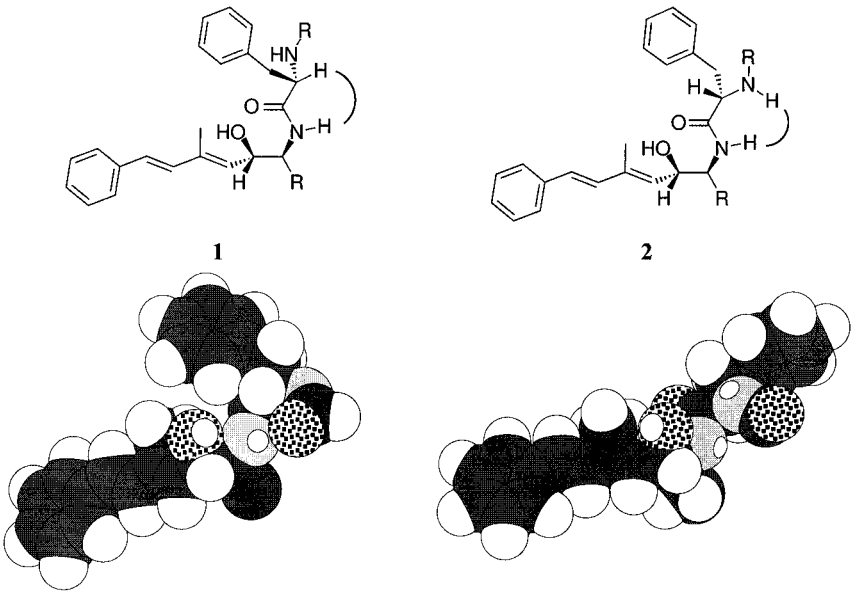


Figure 2. Schematic drawings and space-filling models of Phe and AHMP residues in theopalauamide (**1**) and isotheopalauamide (**2**). Key ROESY correlations are indicated in the top drawings. In the space-filling models, atom types are indicated by patterning: C, dark shading, H, white, N, light shading, O, dark pattern. These models are derived from PCMODEL v 5.0 (MMX) minimized fragments of theopalauamide and isotheopalauamide, restrained on the basis of coupling constants and ROESY data, and are therefore qualitative.

Ozonolysis of Theopalauamide (1) and Isotheopalauamide (2). In separate experiments, samples of peptides **1**, **2**, and **3** (1.2 mg of each) were dissolved in MeOH (0.5 mL) and a stream of ozone in oxygen was bubbled through the cooled solution at −78 °C for 15 min. To the resulting ozonides were added 50% H_2O_2 (10 drops), and the mixture was allowed to stand at room temperature for 1 h. The solvent was removed under a stream of nitrogen, and the ozonolysis products were hydrolyzed and derivatized as described above.

Hydrogenation of 1 and 3 for GC-MS Analysis. Palladium (5%) on charcoal catalyst (3−10 mg) was added to solutions of peptides **1**, **2**, or **3** (1−10 mg in 5 mL of MeOH), and the solutions were stirred under an atmosphere of hydrogen gas for 24 h. The solutions were filtered through Celite, dried under vacuum, and subjected to the hydrolysis/derivatization sequence described above.

Hydrogenation and Hydrolysis To Produce Lactones 4 and 5. Palladium (5%) on charcoal catalyst (250 mg) was added to a solution of a 1:1 mixture of **1** and **2** (120 mg) in MeOH (10 mL), and the mixture was stirred under an atmosphere of hydrogen gas for 24 h. The product was filtered, and the solvent was evaporated to obtain the de-brominated, tetrahydrogenated compounds as indicated by ^{1}H NMR data. The mixture was dissolved in 6 N HCl (6 mL), and the solution was heated for 15 h at 110 °C. The product was lyophilized, and the residue was partitioned between EtOAc and water. The EtOAc layer was evaporated to obtain an inseparable mixture (5.6 mg) of lactones that was analyzed by ^{1}H NMR spectroscopy without separation.

A solution of lactones in ether (0.1 mL) containing 2,4-dinitrofluorobenzene (14 μL) and TEA (4 μL) was stirred for 2 h at room temperature. The solution was filtered through silica gel using 3:2 EtOAc−hexane as eluant, and the mixture of products was separated by HPLC on silica using 1:1 EtOAc−hexane as eluant to obtain pure samples of lactone **4**, which had identical ^{1}H NMR data to literaure values,[7] and lactone **5**.

Lactone 4: ^{1}H NMR ($CDCl_3$) δ 9.17 (d, 1 H, J = 2.5 Hz), 8.80 (d, 1 H, J = 7.5 Hz), 8.33 (dd, 1 H, J = 9.5, 2.5 Hz), 7.1−7.25 (m, 5 H), 6.79 (d, 1 H, J = 9.5 Hz), 4.83 (dt, 1 H, J = 10, 5 Hz), 4.40 (dddd, 1 H, J = 7.5, 6.5, 5, 3 Hz), 3.04 (dd, 1 H, J = 17.5, 7 Hz), 2.70 (m, 1 H), 2.64 (dd, 1 H, J = 17.5, 3 Hz), 2.56 (m, 1 H), 1.95 (ddd, 1 H, J = 15, 10, 5 Hz), 1.77 (m, 1 H), 1.67 (m, 2 H), 1.43 (ddd, 1 H, J = 15, 10, 5 Hz), 1.02 (d, 3 H, J = 6.5 Hz).

Lactone 5: ^{1}H NMR ($CDCl_3$) δ 9.17 (d, 1 H, J = 2.5 Hz), 8.81 (d, 1 H, J = 7.5 Hz), 8.33 (dd, 1 H, J = 9.5, 2.5 Hz), 7.1−7.3 (m, 5 H), 6.80 (d, 1 H, J = 9.5 Hz), 4.83 (dt, 1 H, J = 9, 5 Hz), 4.44 (dddd, 1 H, J = 7.5, 6.5, 5, 3 Hz), 3.04 (dd, 1 H, J = 17.5, 6.5 Hz), 2.70 (m, 1 H), 2.66 (dd, 1 H, J = 17.5, 3 Hz), 2.54 (m, 1 H), 1.88 (m, 1 H), 1.73 (m, 1 H), 1.69 (m, 2 H), 1.48 (m, 1 H), 1.07 (d, 3 H, J = 6.5 Hz).

Acknowledgment. The specimen from Mozambique was collected by Dr. Brad Carté, National University of Singapore, and Dr. Philip Coetzee, University of Port Elizabeth, and was provided to us by Dr. Mike Davies-Coleman, Rhodes University. We also thank the Republic of Palau and the State of Koror for Marine Research Permits. We thank Dr. Jay Siegel for his valuable insights concerning stereochemistry. This research was supported by grants from the National Science Foundation (CHE95-27064), the National Institutes of Health (CA 49084), and the California Sea Grant College Program (NOAA NA36RG0537, project R/MP 60). The Varian Inova 300 MHz NMR spectrometer was purchased using a grant from the National Science Foundation (CHE95-23507).

Supporting Information Available: ^{1}H and ^{13}C NMR and COSY spectra of theopalauamide (**1**) and isotheopalauamide (**2**), an expansion of the ^{1}H NMR spectrum showing the signals due to galactose, and expansions of the ROESY spectra that show the differences between **1** and **2** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9718455