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Axinellin C, a proline-rich cyclic octapeptide isolated from the Fijian marine sponge *Stylotella aurantium*

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Abstract—The structure of a new cyclic octapeptide, axinellin C, (cyclo[Thr[1]-Val[2]-Pro[3]-Trp[4]-Pro[5]-Phe[6]-Pro[7]-Leu[8]]), with all-*trans* peptide bond geometry, was elucidated by a combination of 2D NMR methods and tandem mass spectrometry. The solution state conformation was determined by ROE restrained molecular dynamics calculations. The structural features were found to be similar to those of the crystal structure of the cyclic decapeptide, phakellistatin 8, despite differing peptide sequences.

1. Introduction

The conformation of naturally occurring biologically active cyclic peptides has aroused much interest. Nature's use of non-ribosomal cyclic peptides seems to be for several reasons, resistance to in vivo enzymic degradation, greater bioavailability than non-cyclic analogues and the reduction of conformational flexibility.[1] Noteworthy examples are the proline-rich antamanide[2] from the fungus *Amanita phalloides* and the phakellistatins from various marine sponges.[3–7] In these cases the conformation is constrained by the restraints imposed by the restricted ϕ of the proline residues. A comparison between antamanide and phakellistatin 8 indicates that their gross structural features are similar despite differing amino acid sequences.[8] In this study we show that the solution structure of an octapeptide, axinellin C, retains some of the structural features present in the crystal structure of the decapeptide phakellistatin 8.

2. Collection and isolation

A Fijian collection of the marine sponge *Stylotella aurantium* (Order Halichondrida; Family Halichondriidae) was freeze-dried and subjected to an extraction and partitioning procedure previously described.[9] The dichloromethane extract gave ID_{50}s of 0.47 μg/mL for A2780 ovarian tumour cells and 0.45 μg/mL for K562 leukæmia cancer cells. Sephadex size exclusion chromatography followed by reversed-phase HPLC yielded the new compound axinellin C (**1**), as well as the known compounds pseudoaxinellin,[10] phakellistatin 2[3] and a new conformer of phakellistatin 2. Also isolated was a new heptapeptide, wainunuamide (cyclo[Phe[1]-*trans*-Pro[2]-His[3]-*trans*-Pro[4]-*cis*-Pro[5]-Gly[6]-Leu[7]]).[11]

3. Structure determination

Axinellin C (**1**) showed a molecular ion peak at 938.5096 $[M+H]^+$ Δ 4.4 mmu from calculated for $C_{50}H_{68}N_9O_9$ and requiring 22 degrees of unsaturation. The ^{13}C and DEPT-135 spectrum of axinellin C exhibited eight amide carbonyls and eight α-methine carbons. The amino acid composition was determined after in-depth analysis of ^1H, ^{13}C and 2D NMR data (Table 1): three prolines, one each of phenylalanine, leucine, valine, threonine and tryptophan. HMBC correlations confirmed the assignments of these amino acids. The amino acid composition was confirmed by HPLC analysis of the acid hydrolysate.

The amino acid sequence of axinellin C was determined by a combined approach of 2D NMR and electrospray tandem mass spectrometry techniques. The ROESY spectrum of this compound showed the following cross-peaks: Thr[1]-Hα/Leu[8]-Hα, Phe[6]-Hα/Pro[7]-$H\delta_A$,Pro[7]-$H\delta_B$ and Val[2]-Hα/Pro[3]-$H\delta_A$,Pro[3]-$H\delta_B$ indicating the following sequences: Val[2]-Pro[3], Phe[6]-Pro[7] and Leu[8]-Thr[1]. There were also ROESY cross-peaks between Trp[4]-Hα or Pro[5]-Hα and Pro[5]-$H\delta_A$,Pro[5]-$H\delta_B$ which were difficult to assign due to overlap of the Trp[4]-Hα and Pro[5]-Hα signals. A ROESY cross-peak, however between Phe[6]-H4 and Pro[5]-$H\delta_B$ helped place the remaining proline at position 5 in between the tryptophan and phenylalanine at positions 4 and

Keywords: natural products; amino acids; peptides; *Stylotella aurantium*; sponge; nuclear magnetic resonance; mass spectrometry; restrained molecular dynamics; solution conformation.

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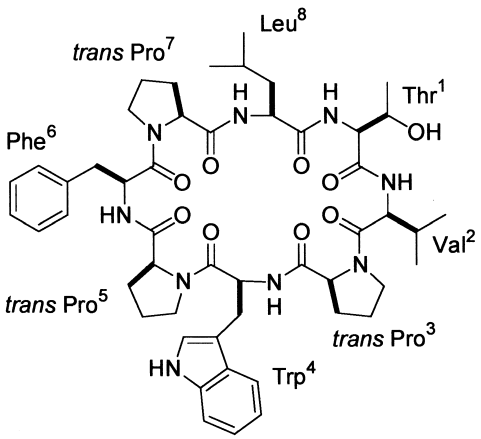
Table 1. ^{1}H (400 MHz, δ (ppm), proton count, multiplicity, J (Hz)), ^{13}C (100 MHz, δ (ppm), multiplicity), and 2D NMR data in CD_3OD for axinellin C (**1**)

Residue	Atom	^{13}C	^{1}H	COSY	HMBC CH
Thr[1]	α	58.1 (d)	4.97 (1H, dd, 3.2, 8.4)	H1β, 1NH	1γCH_3
	β	68.4 (d)	4.31 (1H, m)	H1α, 1γCH_3	1γCH_3
	γCH_3	19.4 (q)	1.19 (3H, d, 6.4)	H1β	
	CO	171.0 (s)			H1α
	NH		7.72 (1H, d, 6.8)	H1α	
Val[2]	α	58.2 (d)	4.66 (1H, d, 8.8)	H2β, 2NH	
	β	29.7 (d)	2.33 (m)	H2α, 2γ_1CH_3, 2γ_2CH_3	2γ_2CH_3, H2α
	γ_1CH_3	20.3 (q)	0.93 (3H, d, 6.8)	H2β	2γ_1CH_3, H2α
	γ_2CH_3	18.7 (q)	0.84 (3H, 8.0)		2γ_1CH_3, H2α
	CO	171.4 (s)			H2α
	NH		7.74 (1H, d, 7.9)	H2α	
Pro[3]	α	62.3 (d)	4.14 (1H, dd, 7.6)		H3α, H3δ_A
	β	30.7 (t)	A. 2.24 (1H, m)	H3β_B	
			B. 1.81 (1H, m)	H3β_A	
	γ	25.8 (t)	A. 2.07 (1H, m)	H3γ_B	H3α
			B. 1.90 (1H, m)	H3γ_A	
	δ	49.2 (t)	3.95 (1H, m)		
			3.68 (1H, m)		
	CO	172.4 (s)			H3α
Trp[4]	α	56.8 (d)	4.34 (1H, m)	H4β_B, 4NH	H4β_A
	β	25.8 (t)	A. 3.55 (1H, m)	H4β_B	
			B 3.39 (1H, m)	H4α, H4β_A	
	2	124.9	6.96 (1H, s)		H4β_A, H4(2)
	3	110.6 (s)			H4β_A, H4(2)
	4	128.3 (s)			H4(2)
	5	118.7 (d)	7.55 (1H, m)	H4(7)	H4(7)
	6	119.7 (d)	7.06 (1H, m)		H4(8)
	7	122.3 (d)	7.09 (1H, m)	4NH(indole), H4(5)	H4(5)
	8	112.3 (d)	7.25 (1H, m)		H4(6)
	9	137.8 (s)			H4(2), H4(5), H4(7)
	CO	173.5 (s)			H4α
	NH(indole)		8.06 (1H, s)	H4(7)	
	NH		7.32 (1H, obs)	H4α	
Pro[5]	α	60.9 (d)	4.34 (1H, m)	H5γ_B	
	β	29.9 (d)	A. 2.03 (1H, m)	H5γ_B	H5α, H5δ_A
			B. 1.40 (1H, m)	H5α	
	γ	25.5 (t)	A. 1.65 (1H, m)	H5γ_B	
			B. 1.40 (1H, m)	H5β_A, H5γ_A	
	δ	48.4 (t)	A. 3.62 (1H, m)	H5δ_B	
			B. 3.28 (1H, m)	H5δ_A	
	CO	173.6 (s)			H5α
Phe[6]	α	52.8 (d)	4.56 (1H, dd, 9.6, 4.0)	H6β_A, H6β_B, 6NH	H6β_A
	β	37.0 (t)	A. 2.62 (1H, m)	H6α, H6β_B	
			B. 2.34 (1H, m)	H6α, H6β_A	
	1	138.4 (s)			H6β_A, H6(3/5)
	2/6	130.0 (d)	7.12 (2H, m)	H6(3/5)	H6β_B, H6(4), H6(2/6)
	3/5	129.0 (d)	7.27 (2H, m)	H6(2/6)	H6(3/5)
	4	127.4 (d)	7.18 (1H, m)		H6(2/6), H6(4)
	CO	172.5 (s)			H6α
	NH		8.22 (1H, d, 7.9)	6Hα	
Pro[7]	α	62.6 (d)	3.94 (1H, m)		H7β_A, H7β_B
	β	30.1 (t)	A. 2.00 (1H, m)	H7β_B	
			B. 1.78 (1H, m)	H7β_A	
	γ	25.2 (t)	A. 1.64 (1H, m)		H7α
			B. 1.39 (1H, m)		
	δ	48.4 (t)	A. 3.96 (1H, m)	H7δ_B	
			B. 3.55 (1H, m)	H7δ_A	
	CO	173.2 (s)			
Leu[8]	α	55.0 (d)	3.66 (1H, m)	8HN	H8β_A
	β	36.0 (t)	A. 2.34 (1H, m)	H8β_B	H8β_A, 8δ_1CH_3, 8δ_2CH_3
			B. 1.72 (1H, m)	H8β_A, H5γ	
	γ	26.0 (t)	1.52 (1H, m)	H5β_B, 8δ_2CH_3	8δ_1CH_3, 8δ_2CH_3
	δ_1CH_3	23.5 (q)	0.91 (3H, d, 6.8)		8δ_1CH_3
	δ_2CH_3	20.8 (q)	0.89 (3H, d, 6.8)	H8γ	8δ_2CH_3, H8β_A
	CO	173.5 (s)			H8α
	NH		8.78 (1H, d, 8.4)	8Hα	



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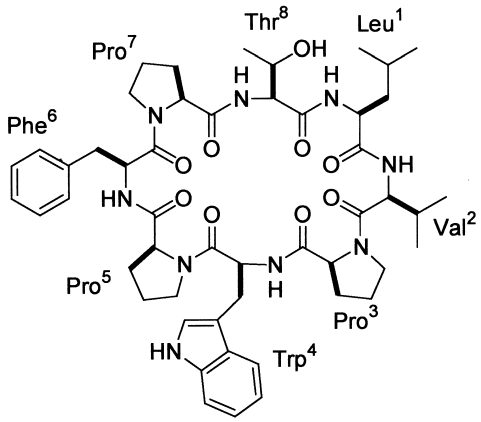

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6, respectively. Long range HMBC correlations were not very informative in terms of sequencing due to extreme overlap of NMR signals in the region of the α-protons in the spectrum but allowed the assignment of the amide carbonyl groups.

The complete sequence of axinellin C was deduced on the basis of the results of ESI-MS^n experiments. The MS spectrum of axinellin C contained signals at *m/z* 938 $[M+H]^+$, and *m/z* 960 $[M+Na]^+$. The ESI-MS^n spectra of axinellin C indicated the preferential formation of *m/z* 920 due to loss of OH of Threonine (Thr) as water before ring opening at any one of the three proline amide bonds. These gave rise to a complex series of fragmentation ions which made interpretation difficult. A few fragmentation pathways emerged (Fig. 1). One started with the opening of the macrocycle (after loss of H_2O) at the Pro^3 amide bond and loss of Pro^3-Trp^4-Pro^5+2H leaving *m/z* 538 corresponding to the sequence Phe^6-Pro^7-Leu^8-Thr^1(-H_2O)-Val^2. The next fragmentation was the loss of Phe^6 leaving *m/z* 391. The formation of *m/z* 294 and *m/z* 181 were due to the loss of Pro^7 and Leu^8, respectively, leaving *m/z* 181 due to Thr^1(-H_2O)-Val^2 which indicated that the suggested sequence was correct. The formation of *m/z* 682 in another pathway corresponded to the sequence Thr^1(-H_2O)-Val^2-Pro^3-Trp^4-Pro^5-Phe^6 which suggested that Phe^6 was adjacently connected to two prolines. This is important as no Pro^5-Phe^6-Pro^7 fragment was observed in the ESI-MS^n spectrum due to the Pro-directed fragmentation pathway favored by this compound. The structure was concluded to be cyclo(Thr^1-Val^2-Pro^3-Trp^4-Pro^5-Phe^6-Pro^7-Leu^8).

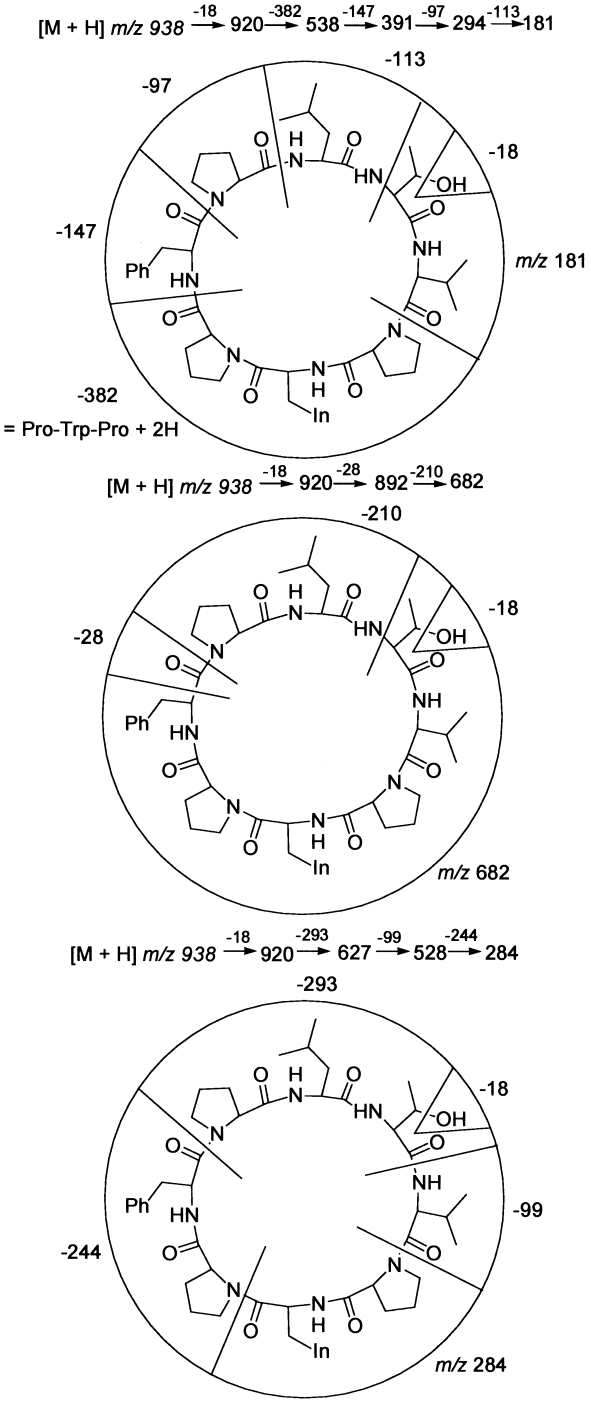

Figure 1. Major fragmentation pathways of **1** in ESI-MS^n.

The last structural feature to be assigned was the geometry of the peptidic linkages at the proline residues. NMR data of axinellin C indicated that all the proline peptide bonds were *trans* as shown by the small difference of ^{13}C NMR chemical shifts of Pro$^3\Delta\delta_{C\beta-C\gamma}$=4.9, Pro$^5\Delta\delta_{C\beta-C\gamma}$=4.4,

Table 2. ROE restraints (w, m, s) for axinellin C (**1**) in CD_3OD

From		To			From		To		
1	Hα	1	γCH_3	m	5	$H\delta_A$	5	$H\delta_B$	s
1	Hα	1	Hβ	m	5	$H\delta_A$	5	$H\gamma_A$	m
1	Hβ	1	γCH_3	m	5	$H\delta_B$	5	$H\gamma_B$	w
1	γCH_3	2	γ_1CH_3	w	5	$H\delta_B$	5	$H\beta_B$	w
1	γCH_3	6	$H\beta_B$	w	5	$H\delta_B$	8	$H\beta_B$	w
2	Hα	2	γ_1CH_3	m	5	$H\delta_B$	8	δ_1CH_3	w
2	Hα	2	γ_2CH_3	m	5	$H\delta_B$	8	δ_2CH_3	w
2	Hα	3	$H\delta_A$	m	5	$H\gamma_A$	5	$H\gamma_B$	s
2	Hα	3	$H\delta_B$	w	6	Hα	7	$H\delta_A$	m
2	Hβ	3	$H\delta_B$	w	6	Hα	7	$H\delta_B$	w
2	Hβ	3	Hδ	w	6	$H\beta_A$	6	$H\beta_B$	s
2	Hβ	8	$H\beta_B$	w	6	H2/6	1	Hα	w
2	γ_2CH_3	2	Hβ	m	6	H2/6	6	Hα	w
3	Hα	3	$H\beta_A$	m	6	Hζ	5	$H\delta_B$	w
3	Hα	4	$H\beta_A$	w	7	Hα	7	$H\beta_A$	m
3	$H\beta_B$	3	$H\beta_A$	s	7	Hα	7	$H\beta_B$	w
3	$H\delta_B$	2	γ_1CH_3	w	7	Hα	8	δ_2CH_3	w
3	$H\delta_B$	3	$H\delta_A$	s	7	$H\beta_A$	7	$H\beta_B$	s
3	$H\gamma_A$	3	$H\gamma_B$	s	7	$H\delta_A$	7	$H\delta_B$	s
3	$H\gamma_A$	3	$H\delta_B$	m	8	Hα	1	γCH_3	w
3	$H\gamma_B$	3	$H\delta_A$	m	8	Hα	8	δ_2CH_3	m
4	$H\beta_A$	5	$H\beta_A$	w	8	$H\beta_A$	8	δ_2CH_3	m
4	$H\beta_B$	4	Hα	w	8	$H\beta_A$	8	δ_1CH_3	m
4	$H\varepsilon_A$	8	$H\beta_A$	w	8	$H\beta_A$	8	$H\beta_B$	s
4	$H\varepsilon_A$	8	$H\beta_B$	w	8	$H\beta_B$	8	Hα	m
5	Hα	5	$H\gamma_A$	w	8	δ_1CH_3	6	Hζ	w
5	Hα	5	$H\gamma_B$	w	8	δ_2CH_3	8	$H\beta_B$	w
5	Hα	5	$H\beta_B$	w	8	Hγ	8	δ_2CH_3	m
5	$H\beta_A$	5	$H\beta_B$	s	8	Hγ	8	$H\beta_A$	m
5	$H\beta_A$	5	Hα	m					

$Pro^7\Delta\delta_{C\beta-C\gamma}$=4.9).[12,13] Confirmatory ROESY cross-peaks observed between Phe^6-Hα/Pro^7-$H\delta_A$,Pro^7-$H\delta_B$ and Val^2-Hα/Pro^3-$H\delta_A$,Pro^3-$H\delta_B$ supported the *trans* geometry of these proline peptidic linkages.[14] The stereochemistry of all residues was found to be L by chiral TLC on the acid hydrolysate.[15]

4. Solution conformation

Axinellin C is structurally related to axinellin B (**2**), previously isolated and described in the literature.[16] They have similar amino acid composition but differ only in the amino acid sequence in the peptide where the order of

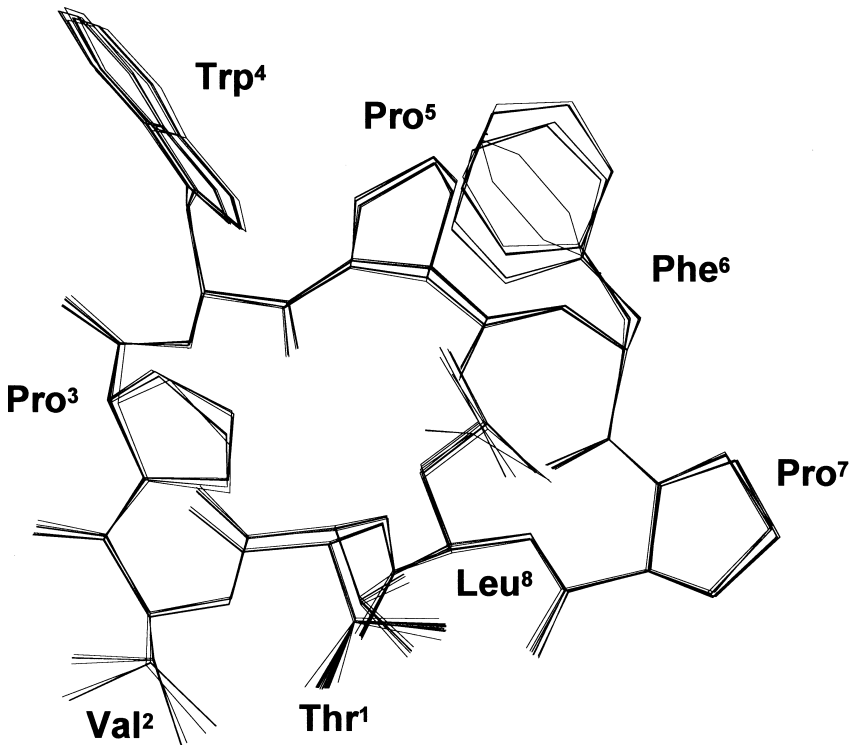

Figure 2. Ensemble of 38 minimum energy structures of axinellin C (**1**) in CD_3OD (heavy atoms only shown).

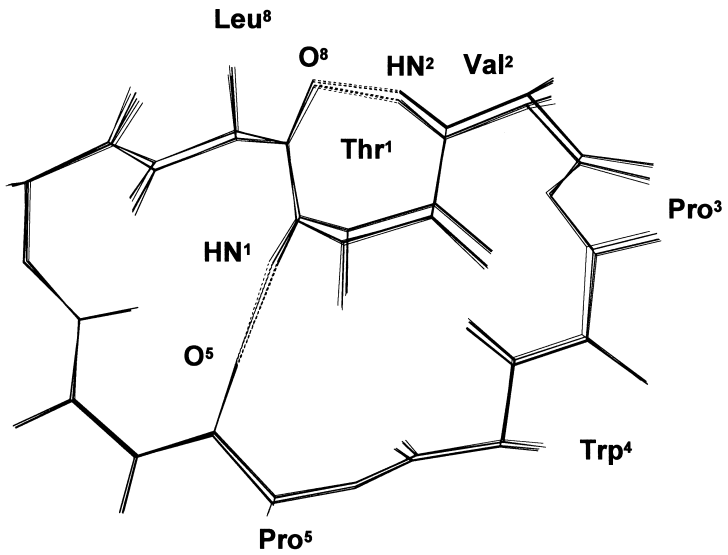

Figure 3. Ensemble of 38 minimum energy structures of axinellin C (**1**) in CD_3OD (backbone only shown) indicating the main hydrogen bonds predicted (dotted lines).

threonine and leucine have been switched. In order to confirm the structure of axinellin C, ROE-restrained molecular dynamics calculations were performed to determine its solution conformation.[9] A total of 59 restraints were obtained and categorized as weak (<5 Å) medium (<3.5 Å) or strong (<2.5 Å) (Table 2). Attempts to use these obtained restraints to model the structure of axinellin B (**2**) failed, and gave structures with high energy, poor geometry, poor refinement and a large number of ROE violations. Conducting the same calculations on the axinellin C (**1**) sequence gave structures with good refinement, a target energy function of 41 kcal/mol, a mean global backbone RMSD of 0.05±0.07 Å for the 38 lowest energy structures (Figs. 2 and 3), and only two minor ROE violations. All ϕ and ψ angles were found to be in the allowed or generously allowed regions of the Ramachandran diagram, as determined by the program Procheck.[17] The absence of restraint violations and good refinement of the consensus structure suggested that sequence **1** proposed for axinellin C was correct.

The calculated structure predicted the presence of two hydrogen bonds: Leu^8-C═O-Val^2-NH and Pro^5-C═O-Thr^1-NH (Fig. 3) which were confirmed by temperature gradients for Val^2-NH and Thr^1-NH of 0.03 and 0.25 ppm/°C, respectively, as compared to the Phe^6-NH value of 0.93 ppm/°C for which no H-bond is predicted. The Leu^8-C═O-Val^2-NH hydrogen bonds constrained a γ-turn for Leu^8-Thr^1-Val^2 with Thr^1 ϕ and ψ angles of 64 and −58°, respectively, compared to ideal angles of 70–85° and −60 to −70°. An additional hydrogen bond was tentatively predicted in the solution conformation between Phe^6-C═O-Thr^1-NH, thus generating a bifurcating hydrogen bond from Thr^1-NH. A type III β-turn was identified encompassing Phe^6-Pro^7-Leu^8-Thr^1 with ϕ and ψ angles for the Pro^7 and Leu^8 residues being −50, −24° and −50, −63°, respectively, close to the ideal −60, −30° required for both these sets of angles.[18]

The solution conformation of **1** showed similarities to that of the X-ray crystal structure of the cyclic decapeptide phakellistatin 8 (cyclo[Pro^1-Pro^2-Ile^3-Phe^4-Val^5-Leu^6-Pro^7-Pro^8-Tyr^9-Ile^{10}]).[8] A similar γ-turn was found, between Ile^3-C═O and Val^5-NH, in addition to which there was an α-turn hydrogen bond between Ile^{10}-C═O and Phe^4-NH similar to that found for **1** between Pro^5-C═O and Thr^1-NH. However, in phakellistatin 8, an additional β-turn was present, constrained by a hydrogen bond from Leu^6-C═O to Tyr^9-NH. In a smaller peptide such as axinellin C, this turn cannot be formed due to structural constraints. A molecular dynamics study on phakellistatin 8 indicates that the Leu^6-C═O to Tyr^9-NH hydrogen bond is lost on going from the crystal structure to aqueous solution.[19]

5. Conclusions

Compound **1** shows weak cytotoxic activity with ID_{50}s of 13.17 and 4.46 μg/mL for A2780 ovarian tumour and K562 leukaemia cancer cells, respectively,[20] indicating that axinellin C is not responsible for the activity discovered in the crude dichloromethane partition fraction. Axinellin C is structurally related to the other cyclohepta- or cyclo-octapeptides characterized by the presence of two or three proline residues an array of apolar residues such as Leu, Ile, Val, and one or two aromatic residues like Trp, Phe or Tyr. The structural features of octapeptide **1** are similar to those of the decapeptides phakellistatin **8** and antamanide despite differing peptide sequences. The conserved nature of this type of fold between species and peptide sequences suggests that this fold may be important for bioactivity.

6. Experimental

6.1. Collection

The sample of *S. aurantium* collection number 9712SD140 was collected in December 1997 at a depth of about 5 m by snorkelling from Cakaulevu reef, in the district of Wainunu, in the island of Vanua Levu, Fiji Islands (17°2.609′; 178°54.694′E). The sample was identified by John Hooper

of the Queensland Centre for Biodiversity and voucher specimens are held at the South Pacific Herbarium, University of the South Pacific, Fiji and at the Marine Natural Products Laboratory, University of Aberdeen, Scotland, UK.

6.2. Extraction and isolation

All sponges were extracted and partitioned by standard procedures as previously described.[23] The dichloromethane fraction was subjected to Sephadex size exclusion chromatography (LH-20) with a mixture of methanol and dichloromethane (50/50) as eluent. Similar fractions were pooled on the basis of TLC analysis. Analysis by ^{1}H and ^{13}C NMR indicated that one pooled fraction contained small peptides. This fraction was given priority for isolation work. Purification was achieved by reversed phase C18 HPLC with a mixture of acetonitrile, water and trifluoroacetic acid (50/50/0.1) as eluent to give axinellin C, pseudoaxinellin (9.2 mg), phakellistatin 2 (6.6 mg) and its conformer (9.5 mg) and wainunuamide (11.2 mg).

Axinellin C (**1**). Colorless oil, 15.0 mg (0.00094% yield). $[\alpha]_D^{25}=-53.1$ (*c* 0.001 MeOH). UV (100% MeOH) λ_{max} 290 (ε 3320). IR (cm^{-1}) 1678, 1550, 1536, 1442, 1203, 1133, 920. Low resolution ESI-MS *m/z* 960.4 $[M+Na]^+$ and high resolution ESI-MS *m/z* 938.5096 Δ 4.4 mmu from that calculated for $C_{50}H_{68}O_9N_9$. NMR data (Table 1)

6.3. Structure calculation

Fifty nine restraints were derived from the T-ROESY spectrum (T_{mix}=300 ms) and classified as weak, medium or strong by contour counting (Table 2). In all cases the lengths of restraints for methyl residues was extended by 0.5 Å to allow for the differential relaxation of methyls. Restrained molecular dynamics calculations were carried out with XPLOR 3.851[21] using a force field with repulsive non-bonded terms. Ab initio simulated annealing calculations (YASAP 3.0: 120 ps total time simulated annealing from 2000 to 100 K, 200 steps minimization) were used to calculate structures from 100 starting conformations with randomized ϕ and ψ angles. From this ensemble, 77 structures were refined using a simulated annealing with slow cooling protocol (600 ps, cooling from 1500 to 100 K, 4000 steps of minimization). The lowest 38 energy structures from the ensemble were selected to represent the final structure. The overlay and display of structures was achieved with Molmol.[22]

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References

1. Wipf, P. *Chem. Rev.* **1995**, *95*, 2115–2134.
2. Kessler, H.; Bats, J. W.; Lautz, J.; Muller, A. *Liebigs Ann. Chem.* **1989**, 913–928.
3. Pettit, G. R.; Tan, R.; Williams, M. D.; Tackett, L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2869–2874.
4. Pettit, G. R.; Cichacz, Z.; Barkoczy, J.; Dorman, A. C.; Herald, D. L.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Brune, D. C.; Cerny, R. L.; Hooper, J. N.; Bakus, G. J. *J. Nat. Prod.* **1993**, *56*, 260–267.
5. Pettit, G. R.; Xu, J.; Cichacz, Z. A.; Williams, M. D.; Chapuis, J. C. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2677–2682.
6. Pettit, G. R.; Xu, J.; Cichacz, Z.; Williams, M. D.; Dorsaz, A. C.; Brune, D. C. *Biorg. Med. Chem. Lett.* **1994**, *4*, 2091–2096.
7. Pettit, G. R.; Tan, R.; Herald, D. L.; Cerny, R. L.; Williams, M. D. *J. Org. Chem.* **1994**, *59*, 1593–1595.
8. Herald, D. L.; Cascarano, G. L.; Pettit, G. R.; Srirangam, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 6962–6973.
9. Morris, L. A.; van den Bosch, J. J. K.; Versluis, K.; Thompson, G. S.; Jaspars, M. *Tetrahedron* **2000**, *56*, 8345–8353.
10. Kong, F.; Burgoyne, D. L.; Andersen, R. J. *Tetrahedron Lett.* **1992**, *33*, 3269–3272.
11. Tabudravu, J. N.; Morris, L. A.; Kettenes van den Bosch, J. J.; Jaspars, M. *Tetrahedron Lett.* **2001**, *42*, 9273–9276.
12. Dorman, D. E.; Borvey, F. A. *J. Org. Chem.* **1973**, *38*, 1719.
13. Dorman, D. E.; Borvey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.
14. McDonald, L. A.; Forster, M. P.; Phillips, D. R.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 4616–4624.
15. Gunther, K.; Martens, J.; Schickedanz, M. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 506.
16. Randazzo, A.; Piaz, F. D.; Orru, S.; Debitus, C.; Roussakis, C.; Pucci, P.; Paloma, L. G. *Eur. J. Org. Chem.* **1998**, 2659–2665.
17. Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. *J. Appl. Crystallogr.* **1993**, *26*, 283–291.
18. Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*. Fasman, G. D., Ed.; Plenum: New York, 1996; p 738.
19. Galzitskaya, O.; Caflisch, A. *J. Mol. Graph. Model.* **1999**, *17*, 19–27.
20. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
21. Brunger, A. T. *X-PLOR a system for X-ray crystallography and NMR 3.851*; Yale University: New Haven, 1993.
22. Koradi, R. M. B.; Wuthrich, K. *J. Mol. Graph.* **1994**, *14*, 51–59.
23. Morris, L. A.; Jaspars, M.; Adamson, K.; Woods, S.; Wallace, H. M. *Tetrahedron* **1998**, *54*, 12953–12958.