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A Dimeric Peptide Alkaloid of a Completely New Type, Anchinopeptolide A, from the Marine Sponge *Anchinoe tenacior*

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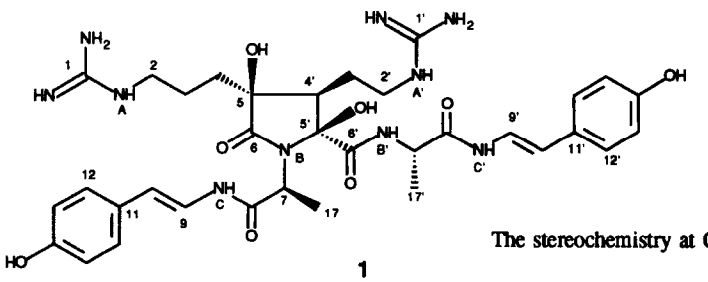
Abstract: Anchinopeptolide A (**1**), a dimeric peptide alkaloid of a completely new type, has been isolated from the marine sponge *Anchinoe tenacior* collected in the Mediterranean sea along the coasts of Tunisia. The structure elucidation of **1**, which include in each half a C-terminal *trans* 4-hydroxystirylamino residue linked to L-alanine, which in turn is attached to an arginine derived 5-guanidino-2-hydroxy-pentanoyl residue, is described.

Over the past 25 years, sponges has proven to be a source of an extraordinary number of metabolites, among which linear and cyclic peptides and depsipeptides are an emerging important class of metabolites with significant biological activity [1]. Most sponge peptides are severely modified, so that only a few "true" peptides exist which posses recognizable amino acid linkages. For example clionamide [2] and four other related linear peptide alkaloids celenamides [3,4], isolated from the sponge *Cliona celata*, possess C-terminal styrylamino residues, previously found only in terrestrial plant alkaloids [5].

We now report the structure elucidation of a dimeric peptide alkaloid, anchinopeptolide A (**1**), present in the sponge *Anchinoe tenacior*, which possess in each half a C-terminal *trans*-4-hydroxystyrylamino residue.



1

The stereochemistry at C-5, C-4' and C-5' is relative

Anchinoe tenacior (Poecilosclerida, Demospongiae) Topsent, an encrusting sponge of a deep blue colour, was collected in the Mediterranean Sea along the coasts of Tunisia. The freshly collected sponge was frozen on site and transferred to our Department over dry ice. Frozen sponge (1.5 Kg fresh) was homogenized in acetone and extracted at room temperature for two days. The acetone filtrate was evaporated in *vacuo* to give a residue that was suspended in water and sequentially extracted with ethyl ether and *n*-butanol. Purification of the *n*-butanol soluble material by sequential application of Sephadex LH-20 (eluent methanol), droplet counter-current chromatography (DCCC; *n*-BuOH-Me_2CO-H_2O, 3 :1 :5, ascending mode, the lower phase was used as stationary phase) and HPLC (C_{18} μ-bondapak; MeOH-H_2O, 7.5:92.5) gave four major related dimeric peptide alkaloids, named anchinopeptolide A (7 mg), B (7.1 mg), C (41 mg), D (35.4 mg). In this paper we describe evidence consistent with structure **1** of anchinopeptolide A.

The fast atom bombardment (FAB) mass spectrum of **1**, $[\alpha]_D$ = -103.6° (MeOH, c = 4), in the presence of trifluoromethanesulphonic acid, gave a pseudomolecular ion m/z 873 $[M + H + CF_3SO_3H]^+$, corresponding to a m.w. of 722 daltons; the infrared (IR) spectrum showed absorption bands due to amide groups (3343, 1700, 1685, 1653 and 1540 cm^{-1} in KBr) and the UV (MeOH) showed absorption at 217 nm, ε 13570 and 284.5 nm, ε 20030, suggesting a styryl chromophore. The ^{13}C-NMR spectrum (CD_3OD, Table) gave 30 signals corresponding to 34 carbons atoms, four of them equivalent (*i.e.* C-12 and C-16, C-12' and C-16'; C-13 and C-15, C-13' and C-15'): two methyls, five methylenes, fifteen methines, and twelve quaternary carbons. Of the quaternary carbons four (δ_C 176.5, 174.3, 171.8 and 169.8) can be assigned to carbonyl amides, two (δ_C 158.6 and 158.7 ppm) have chemical shifts expected for guanidine carbons, four (δ_C 157.5, 157.6, 129.0 and 128.9 ppm) may be assigned to two *p*-hydroxyphenyl residues, and finally the signals at 92.2 and 77.0 ppm may be assigned to hydroxyl-bearing carbons. The ^{1}H-NMR spectrum in DMSO-d_6 disclosed 15 exchangeable signals for two phenolic groups at δ 9.38 s and 9.35 s, two hydroxyl groups at δ 7.49 s and 5.68 s, three amide groups at δ 10.26 d, 9.49 d, 8.48 d and two 1 H signals at 7.90 t and 7.74 t, which along with two 3 H broad signals centered at δ 6.85 and 7.40, indicated the presence of two guanidine groups. This suggested that one remaining amide nitrogen is tertiary. All these data taken together led to the molecular formula $C_{34}H_{46}N_{10}O_8$ for anchinopeptolide A (**1**), which required seventeen sites of unsaturation. Since only sixteen of these sites of unsaturation could be accounted for by functionalities disclosed by NMR spectroscopy (*i.e.* two styryl units, four C=O, and two guanidine groups) it was apparent that **1** must contain one more ring. The ^{1}H-NMR spectrum of **1** displayed resonances at δ 5.86 d (1H, J= 14.6 Hz), and 6.10 d (1H, J= 14.6 Hz), at δ 7.08 (6H overlapping signals, H-9, H-9', H-12, H-16, H-12', H-16'; in CD_3OD these signals are separated giving rise to two overlapping doublets at δ 7.18 (J=14.3 Hz) and 7.19 (J= 14.3 Hz) for the olefinic protons H-9 and H-9', and two overlapping doublets at δ 7.07 (J= 8.0 Hz) and 7.06 (J=8.0 Hz) for the aromatic protons C-12, C-16 and C-12', C-16'), and at δ 10.26 (1H, d, J= 9.8 Hz, exchangeable) and 9.49 (1H, d, J=9.5 Hz, exchangeable), indicative of two *trans* substituted enamides [2-4]. The signals at δ 6.64 (4H, two overlapping doublets with J=7.8 Hz), δ 7.08 (4H, overlapped with the olefinic signals H-9, H-9'), 9.38 (1H, s, exchangeable) and 9.35 (1H, s, exchangeable) suggested that the enamide substituents were *p*-hydroxyphenyl groups. 2D-COSY {1H,1H} and 2D-NOESY (DMSO-d_6) experiments showing cross peaks N_C-H/H-9 and $N_{C'}$-H/H-9' (COSY) and N_C-H/H-10 and $N_{C'}$-H/H-10' (NOESY) confirmed the presence of two C-terminal *trans-p*-hydroxystyrylamino residues. Signals at δ 4.40 (1H, quintet, J=7.5) coupled with methyl doublets at δ 1.36 (J=6.8 Hz) and with the $N_{B'}$-H at δ 8.48 (d, J= 6.8 Hz) indicated an alanyl residue. A second alanyl residue with the nitrogen tertiary was indicated by a 1H quartet at δ 3.88 (J=6.8 Hz) coupled with the methyl doublet at δ 1.41 (J=6.8). Hydrolysis with 6N HCl followed by derivatization with Marfey's reagent and HPLC analysis [6] showed that the alanines had the L configuration. ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectra in DMSO-d_6 clarified the sequences *p*-hydroxystyrylamino/alanines residues by giving cross peaks as indicated in the partial structures shown below.

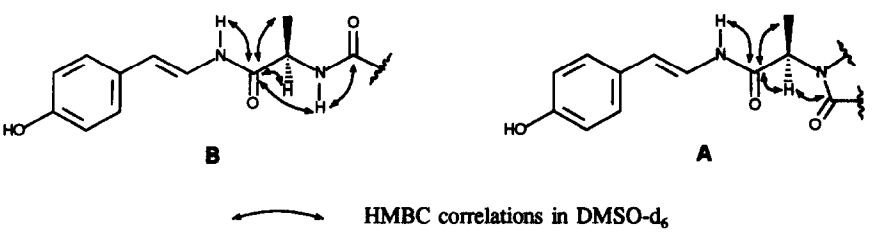


Table 1. ^{1}H and ^{13}C NMR Data of Compound **1**.

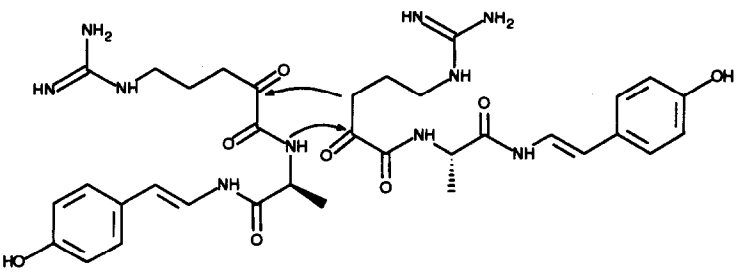
	CD_3OD		DMSO-d_6			CD_3OD		DMSO-d_6	
Position	δ_C	δ_H	δ_C	δ_H	Position	δ_C	δ_H	δ_C	δ_H
1	158.6		157.2		1'	158.7		157.2	
2	42.5	3.19 m	41.3	3.08 2H, m	2'	40.6	3.37 2H, m	38.4	3.22 2H, m
3	24.8	1.48 ,1.85m	23.8	1.28 m, 1.66 m	3'	24.2	2.00 2H, m	23.2	1.77 m, 1.86 m
4	34.4	1.85 m	34.2	1.32 m, 1.57 m	4'	46.1	2.80 t (8.5)	44.8	2.61 t (6.8)
5	77.0		74.9		5'	92.2		90.5	
6	176.5		174.1		6'	174.3		172.6	
7	53.3	4.13 q (7.1)	49.5	3.88 q (6.8)	7'	51.8	4.45 q (7.1)	48.8	4.40 quintet (6.8)
8	169.8		167.6		8'	171.8		169.3	
9	121.1	7.18 d (14.3)	120.8	7.08 m	9'	121.3	7.18 d (14.3)	121.1	7.08 m
10	115.9	6.19 d (14.3)	113.1	6.10 d (14.6)	10'	114.8	6.07 d (14.3)	111.4	5.86 d (14.6)
11	128.9		127.3		11'	129.0		127.3	
12, 16	127.8	7.06 d (8.0)	126.5	7.08 m	12', 16'	127.9	7.07 d (8.0)	126.7	7.08 m
13, 15	116.4	6.65 d (8.0)	115.7	6.64 d (8.0)	13', 15'	116.5	6.66 d (8.0)	115.7	6.65 d (8.0)
14	157.5		156.2		14'	157.6		156.4	
17	14.6	1.59 d (7.1)	14.4	1.41 d (6.8)	17'	17.6	1.53 d (7.1)	17.9	1.36 d (6.8)
N_A-H				7.74 t (4.7)	$N_{A'}$-H				7.90 t (4.7)
					$N_{B'}$ - H				8.48 d (6.8)
N_C-H				9.49 d (9.5)	$N_{C'}$-H				10.26 d (9.8)
5-OH				5.68 s	5'-OH				7.49 s
14-OH				9.35 s	14'-OH				9.38 s
$C(=NH)NH_2$				6.85 3H, broad	$C(=NH)NH_2$				7.40 3H, broad

NMR spectra were recorded on a Bruker AMX-500 spectrometer. The coupling constants are given in Hz and are enclosed in parentheses. ^{1}H assignments were based on {^{1}H-^{1}H} COSY experiments; signals assigned to the phenyl residues can be interchanged between the two units, as well as the broad signals for the guanidine -$C(=NH)NH_2$ protons can be interchanged between the two halfs. ^{13}C assignments were based on hetero-correlation experiments (HETCOR and COLOC in CD_3OD, HMQC and HMBC in DMSO-d_6).

Further analysis of 2D-COSY spectra in DMSO-d_6 verified two-coupled networks: the three methylenic protons on C-2, C-3, C-4 with the guanidino group at C-2 and the NH=(NH_2)C-NH-CH_2-CH_2-CH< system. The observation of a cross peak of H_2-4 to the quaternary C-5 at 74.9 ppm in the COLOC spectrum in CD_3OD supported the connectivity C-4 and C-5. Futher information on the structure was obtained through the analysis of HMBC spectra in DMSO-d_6, which showed key cross peaks of 5'-OH to the quaternary C-5' at 90.5 ppm and to the carbonyl at C-6', at 172.6 ppm, and of H-7 to the quaternary C-5' at 90.5 ppm and to the remaining carbonyl at C-6, at 174.1 ppm. The structure of anchinopeptolide A was, consequently, concluded to be **1**.

The stereochemistry around the 2-pyrrolidone ring was suggested by NOE (NOE difference experiments) between 5-OH/5'-OH, 5-OH/H_2-3' and 5'-OH/H_2-3'. It is to be noted that the molecule exhibited negative NOE effects.

The structure of **1** is a new molecular entity, which might originate biogenetically by dimerization of two halves, each containing 2-oxo-5-guanidino-pentanoyl, L-alanyl and *p*-hydroxystyrylamino residues, as indicated in Scheme 1. The isolation from *Anchinoe tenacior* and structure elucidation of other compounds belonging to this new class of alkaloid as well as the study of their possible biological activity is currently under way in our laboratory.



Scheme 1. Possible biogenetic formation of the five-membered ring of anchinopeptolide A (**1**).

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5) For example, see : a) R. Tschesche, E. Frohberg, H. W. Fehlhober, *Tetrahedron Lett.*, **1968**, 1311. b) J. Marchand, M. Pais, F. Jarreau, *Tetrahedron*, **1969**, 25, 937.
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7) The experiment was optimized for long range couplings with a fixed delay Δ= 60 ms. The low pass J-filter in the experiment to eliminate responses from direct ($^1J_{CH}$) pairs was optimized for 150 Hz.

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