Four New Dimeric Peptide Alkaloids, Anchinopeptolides B-D, and Cycloanchinopeptolide C, Congeners of Anchinopeptolide A, from the Mediterranean Marine Sponge Anchinoe tenacior

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FOUR NEW DIMERIC PEPTIDE ALKALOIDS, ANCHINOPEPTOLIDES B–D, AND CYCLOANCHINOPEPTOLIDE C, CONGENERS OF ANCHINOPEPTOLIDE A, FROM THE MEDITERRANEAN MARINE SPONGE *ANCHINOE TENACIOR*

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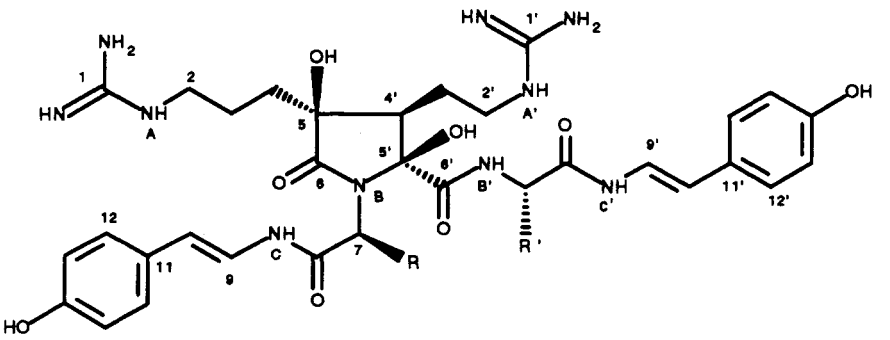
ABSTRACT.—Following the characterization of anchinopeptolide A [**1**], three new congeneric dimeric peptide alkaloids, named anchinopeptolides B [**2**], C [**3**], and D [**4**], have been isolated from the Mediterranean sponge *Anchinoe tenacior*. A fourth compound, cycloanchinopeptolide C [**5**], which is related to anchinopeptolide C [**3**] by a head-to-head intramolecular [2+2] cyclo-addition reaction, has also been isolated. The structures of these peptide alkaloids have been elucidated on the basis of spectral evidence. Anchinopeptolides bind to the somatostatin, human B2 bradykinin, and neuropeptide Y receptors.

Sponges, expecially those belonging to the order Lithistida (e.g., in the genera *Theonella* and *Discodermia*), are known to contain highly bioactive linear and cyclic peptides, which often have unusual amino acids (1,2). During the course of our investigation of new active substances from marine organisms we isolated four dimeric peptide alkaloids, anchinopeptolides A [**1**], B [**2**], C [**3**], and D [**4**]. On the basis of spectral data, we elucidated the structure of anchinopeptolide A as **1**, which contains in each half a *C*-terminal *trans*-4-hydroxystyrylamino residue linked to L-alanine, which in turn is attached to a 5-guanidino-2-hydroxypentanoyl residue, probably derived from arginine (3). Further investigation on the *n*-BuOH soluble portion of the Me_2CO extract has led to the isolation of another related dimeric peptide alkaloid, cycloanchinopeptolide C [**5**]. This paper deals with the structural elucidation of these congeneric peptide alkaloids, i.e., anchinopeptolides B [**2**], C [**3**], and D [**4**], and cycloanchinopeptolide C [**5**].

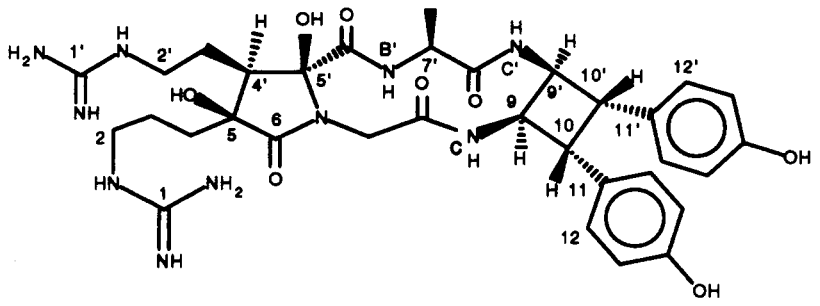
RESULTS AND DISCUSSION

The marine sponge *Anchinoe tenacior* (Demospongiae, Poecilosclerida, Anchinoideae)



1 R=R′=Me
2 R=Me, R′=H
3 R=H, R′=Me
4 R=H, R′=H

(The stereochemistry at C-5, C-4′, and C-5′ is relative.)



5

(The stereochemistry at C-5, C-4′, C-5′, and around the cyclobutane ring is relative.)

Topsent (1.5 kg), collected in June 1991 along the coast of Tunisia, was extracted with Me_2CO. Sephadex LH-20 cc (eluent MeOH) of the *n*-BuOH-soluble portion of the Me_2CO extract, followed by droplet counter-current chromatography in *n*-BuOH-Me_2CO-H_2O, 3:1:5, and reversed-phase hplc developed with MeOH/H_2O, provided anchinopeptolides A (**1**, 7 mg), B (**2**, 7.1 mg), C (**3**, 41 mg), and D (**4**, 35.4 mg), and cycloanchinopeptolide C (**5**, 4.1 mg).

Anchinopeptolide D [**4**], the most polar of the five metabolites, gave by fabms in the presence of CF_3SO_3H a pseudomolecular ion at *m/z* 845 $[M+H+CF_3SO_3H]^+$, corresponding to a mol wt of 694 daltons, which was 28 mass units fewer than anchinopeptolide A [**1**]. The ^{1}H-nmr spectra in two solvents (Tables 1 and 2) were very close to those of anchinopeptolide A [**1**], except for signals assignable to the alanyl methyl groups, which were missing in **4**, and for signals assigned to the α-methine protons of the alanyl residues, which were replaced in **4** by two sets of methylene proton signals resonating in DMSO-d_6 at δ 3.87 (br d, 2H) and at 3.74 (1H, d, J=16.6 Hz) through 4.02 (1H, d, J=16.6 Hz). Further, the exchangeable doublet at δ 8.48 assigned to $N_{B'}$-H in **1** was replaced in **4** by a triplet at δ 8.73. Thus, anchinopeptolide D [**4**] was presumed to be related to **1** by replacing the alanyl residues with glycyl ones. This was confirmed by 2D nmr spectral data, inclusive of COSY, COLOC, and NOESY. Evaluation of the 2D-COSY spectrum in DMSO-d_6 confirmed the presence of two coupled networks: the methylene protons at C-2, C-3, and C-4 with the guanidino group at C-2 and the HN=(NH_2)C-NH-CH_2-CH_2-CH- system. The observation of a cross-peak of H_2-4 (δ 1.60 m–1.64 m) to the quaternary C-5 (δ_C 74.5 ppm) in the COLOC (correlation spectroscopy via long-range coupling) spectrum verified the connectivity of C-4 and C-5. Continuing the analysis of the COLOC spectrum, it was observed that the signal at 175.4 ppm exhibited two cross-peaks to the glycine H-7_a signal at δ 3.74 (d, J=16.6 Hz) and to the 5-OH signal at δ 5.59 s, and is assignable to the C-6 carbonyl carbon, whereas the signal at 171.1 ppm exhibited two cross-peaks to the H_2-7′ signal at δ 3.87 (br) and to the 5′-OH signal at δ 7.15 s, and is assignable to the C-6′ carbonyl carbon. A cross-peak between the glycine H-7_b signal at δ 4.02 (d, J=16.6 Hz) and the C-5′ carbon at 89.3 ppm was also recognized in the COLOC spectrum. These cross-peaks can be well explained by the structure **4** (Figure 1). NOESY (nuclear Overhauser and exchange spectroscopy) was used to determine the steric configurations of substituents on the 2-pyrrolidone ring. In the NOESY spectrum, there was a cross-peak between the 5-OH and the 5′-OH protons, indicating that the two hydroxyl groups are cis to each other. In addition, the presence of cross-peaks between the 3′-methylene protons and both the OH protons showed that the side-chains at C-5 and C-4′ are trans to each other. Further, the cross-peaks N_CH/H_2-7, N_C-H/H-10, $N_{C'}$-H/H_2-7′, $N_{C'}$-H/H-10′, H-9/H-12 and H-16, H-9′/H-12′ and H-16′ were also recognized, confirming the sequences of the *p*-

TABLE 1. ^{13}C- and ^{1}H-Nmr Spectral Data of Compounds **2**–**5** in CD_3OD.[a]

Position	Compound							
	2		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	158.8		158.4		158.4		158.6	
2	42.5	3.17 t (6.8)	42.3	3.25 t (6.8)	42.6	3.24 t (6.8)	42.5	3.28 t (6.8)
3	24.8	1.49 m, 1.86 m	24.5	1.58 m, 1.88 m	24.5	1.58 m, 1.86 m	24.2	1.94 m
4	34.2	1.86 m	34.7	1.88 m	34.4	1.86 m	34.8	1.90 m
5	77.0		76.5		76.5		76.5	
6	176.6		177.8		177.7		178.3	
7	53.6	4.04 q (6.8)	44.9	4.00 d, 4.20 d (17.0)	44.9	3.93 d, 4.26 d (16.6)	44.2	4.03 d, 4.17 d (17.3)
8	169.8		167.8		167.8		170.2	
9	121.1	7.23 d (14.6)	121.0	7.24 d (14.6)	120.8	7.28 d (14.6)	52.7	4.80 dd (4.1, 7.2)
10	115.9	6.08 d (14.6)	116.0	6.21 d (14.6)	115.9	6.22 d (14.6)	47.5	3.89 dd (4.1, 10.5)
11	129.0		128.8		128.7		127.9	
12,16	127.8	7.15 d (8.5)	127.8	7.15 d (8.5)	127.7	7.18 d (8.5)	130.3	6.85 d (8.5)
13,15	116.6	6.74 d (8.5)	116.4	6.72 d (8.5)	116.4	6.74 d (8.5)	115.9	6.59 d (8.5)
14	157.6		157.6		157.5		156.8	
17	14.6	1.58 d (6.8)						
1′	158.8		158.5		158.4		158.6	
2′	41.0	3.27 m, 3.49 m	40.4	3.31 m	40.6	3.29 m, 3.49 m	40.6	3.24 m
3′	23.9	2.02 m, 2.11 m	24.1	1.94 m, 2.02 m	24.1	1.92 m, 2.10 m	24.4	1.58 m, 1.85 m
4′	47.0	2.7 dd (5.4, 6.1)	45.9	2.79 br t (6.8)	46.4	2.74 dd (4.5, 5.1)	44.0	3.20 t (5.0)
5′	92.6		90.7		90.1		90.6	
6′	174.5		172.5		173.4		173.8	
7′	43.3	3.97 d, 4.29 d (16.6)	51.3	4.41 q (6.8)	43.3	3.96 d, 4.16 d (17.0)	50.7	4.64 q (6.8)
8′	168.2		171.9		168.1		173.4	
9′	121.5	7.29 d (14.6)	120.6	7.24 d (14.6)	120.6	7.25 d (14.6)	52.7	4.76 dd (3.0, 7.2)
10′	114.7	6.23 d (14.6)	115.6	6.25 d (14.6)	115.4	6.20 d (14.6)	48.3	4.06 dd (3.0, 10.5)
11′	129.0		128.6		128.8		127.9	
12′,16′	127.9	7.19 d (8.5)	127.7	7.13 d (8.5)	127.7	7.18 d (8.5)	130.0	6.73 d (8.5)
13′,15′	116.6	6.71 d (8.5)	116.4	6.72 d (8.5)	116.4	6.74 d (8.5)	115.8	6.55 d (8.5)
14′	157.6		157.4		157.6		156.9	
17′			17.6	1.50 d (6.8)			14.6	1.43 d (6.8)

[a]Nmr spectra were recorded on a Bruker AMX-500 spectrometer. The coupling constants are given in Hz and are enclosed in parentheses. Signals assigned to styryl residues can be interchanged between the two units, and the broad signals for the guanidine $-C(=NH)NH_2$ protons can be interchanged between the two halves. In **4** and **5**, ^{13}C-nmr assignments were based on HETCOR and COLOC experiments.

hydroxystyrylamino/glycine residues. Thus, the structure of anchinopeptolide D was determined as **4**, which is related to **1** by the presence of two glycine residues instead of two alanine residues. Interestingly, the ^{1}H-nmr signal for H-4′ in CD_3OD, which was a broad triplet in **1**, was a sharp double doublet (J=4.5 and 5.1 Hz) in **4**.

Anchinopeptolide B [**2**] was smaller than anchinopeptolide A [**1**] by 14 mass units, with a fabms fragment peak at *m/z* 859 $[M+H+CF_3SO_3H]^+$, corresponding to a mol wt of 708 daltons. ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) revealed that anchinopeptolide B [**2**] is related to **1** by replacement of one alanyl residue with a glycyl unit. The ^{1}H-nmr spectrum in DMSO-d_6 showed the glycyl α-methylene protons as two 1H double doublets at δ 3.98 (J=17.1 and 6.1 Hz) and 3.91 (J=17.1 and 6.1 Hz) coupled with the glycyl amide proton which resonated as a triplet at δ 8.92 (J=6.1 Hz), whereas the alanyl methine proton was observed as a quartet at δ 3.86 (J=6.8 Hz) coupled only with the methyl doublet at δ 1.42. This established the relative positions of the alanyl and the glycyl residues in **2**. Once again the ^{1}H-nmr signal for H-4 was a sharp double doublet in **4**, both having the side-chain at C-5′ containing the glycyl residue instead of the alanyl one.

TABLE 2. ^{13}C- and ^{1}H-Nmr Spectral Data of Compounds **2–5** in DMSO-d_6.[a]

Position	Compound							
	2		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	157.1		156.9		157.0		157.7	
2	41.0	3.13 m	40.9	3.13 m	41.2	3.11 m	41.7	3.10 br
3	23.0	1.65 m	23.1	1.65 m	23.2	1.35, 1.60 m	24.1	1.63 m
4	34.1	1.62 m	34.1	1.64 m	34.2	1.60, 1.64 m	35.0	1.63 m
5	74.8		74.4		74.5		75.1	
6	174.1		175.3		175.4		172.6	
7	51.3	3.86 q (6.8)	44.1	3.71 d, 3.97 d (16.6)	44.2	3.74 d, 4.02 d (16.6)	44.2	3.85 br
8	167.6		165.9		166.0		168.4	
9	121.0	7.14 dd (10.5, 14.2)	120.6	7.12 dd (10.5, 14.6)	120.5	7.15 dd (10.5, 14.6)	52.7	4.53 m
10	112.9	6.10 d (14.2)	113.3	6.22 d (14.6)	113.4	6.21 d (14.6)	47.5	3.85 m
11	127.2		127.0		127.0		129.3	
12,16	126.5	7.14 d (8.5)	126.4	7.12 d (8.5)	126.5	7.15 d (8.5)	129.5	6.65 d (8.5)
13,15	115.8	6.67 d (8.5)	115.5	6.68 d (8.5)	115.6	6.69 d (8.5)	116.6	6.50 d (8.5)
14	156.4		156.2		156.3		156.1	
17	14.2	1.42 d (6.8)						
N_A-H		7.67 br		7.73 br		7.65 br		7.82 br
N_C-H		9.59 d (10.5)		10.11 d (10.5)		10.15 d (10.2)		7.32 d (8.2)
5-OH		5.70 s		5.61 s		5.59 s		5.48 s
14-OH		9.41 s		9.42 s		9.43 s		9.15 s
$C(=NH)NH_2$		6.91, 3H, br		6.95, 3H, br		6.91, 3H, br		6.90, 3H, br
1′	157.1		157.0		157.0		157.6	
2′	40.3	3.13 m	39.0	3.13 m	39.3	3.10, 3.23 br	41.7	3.10 br
3′	23.5	1.87 m	23.5	1.84 m	23.6	1.84, 1.92 m	24.3	1.75 m
4′	45.0	2.63 m	44.0	2.65 t (7.1)	44.6	2.59 dd (4.8, 5.1)	43.2	3.02 m
5′	90.4		89.1		89.3		89.4	
6′	170.3		170.3		171.1		171.6	
7′	42.5	3.91 dd, 3.98 dd (6.1, 17.1)	49.2	4.30 quint. (6.8)	42.5	3.87 br	49.7	4.42 quint. (6.8)
8′	166.3		169.6		166.5		174.4	
9′	121.0	7.14 dd (10.2, 14.2)	120.2	7.12 dd (10.3, 14.6)	120.2	7.15 dd (10.2, 14.6)	49.4	4.55 m
10′	111.3	5.87 d (14.2)	112.7	6.13 d (14.6)	112.5	6.09 d (14.6)	46.6	3.85 br
11′	127.2		126.9		127.1		129.3	
12′,16′	126.7	7.14 d (8.5)	126.4	7.12 d (8.5)	126.4	7.15 d (8.5)	129.7	6.73 d (8.5)
13′,15′	115.8	6.67 d (8.5)	115.5	6.68 d (8.5)	115.6	6.69 d (8.5)	115.6	6.53 d (8.5)
14′	156.4		156.1		156.4		156.1	
17′			17.5	1.34 d (6.8)			15.1	1.26 d (6.8)
N_A-H		7.86 br		7.82 br		7.77 br		7.78 br
N_B-H		8.92 t (6.1)		8.34 d (6.8)		8.73 br t (5.5)		8.67 d (6.8)
N_C-H		10.38 d (10.2)		10.27 d (10.3)		10.32 d (10.2)		7.91 d (8.8)
5′-OH		7.43 s		7.12 s		7.05 s		7.46 s
14′-OH		9.87 s		9.40 s		9.41 s		9.17 s
$C(=NH)NH_2$		7.35, 3H, br		7.42, 3H, br		7.38, 3H, br		7.44, 3H, br

[a]Nmr spectra were recorded on a Bruker AMX-500 spectrometer. The coupling constants are given in Hz and are enclosed in parentheses. In **2–4**, signals assigned to the styryl residues can be interchanged between the two units. In **2–5** the broad signals for the guanidine -$C(=NH)NH_2$ protons can be interchanged between the two halves. In **3** and **4**, ^{13}C-nmr assignments were based on HETCOR and COLOC experiments.

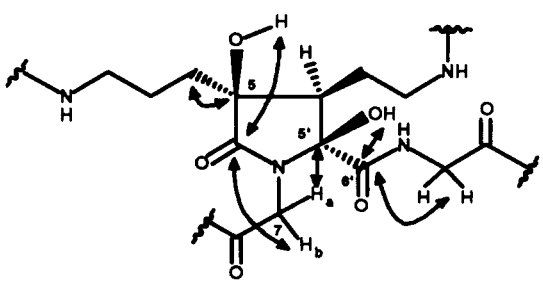


FIGURE 1. Key COLOC Correlations Around the 2-Pyrrolidone Ring of **4** in DMSO-d_6.

Anchinopeptolide C [**3**], the major peptide alkaloid among the five metabolites, is isomeric with anchinopeptolide B [**2**], and showed in the fabms a fragment peak at *m/z* 859 $[M+H+CF_3SO_3H]^+$, corresponding to a mol wt of 708 daltons. The ^{1}H-nmr spectra (Tables 1 and 2) were almost superimposable with those of anchinopeptolide B [**2**], except for signals assignable to glycyl and alanyl residues which in **3** in DMSO-d_6 appeared as two sharp 1H doublets at δ 3.97 and 3.71 (J=16.6 Hz) (Gly) and as a quintet at δ 4.30 (J=7.0 Hz) coupled with the methyl doublet at δ 1.34 and with the amide proton at δ 8.34 (d, J=6.8 Hz) (Ala). This established the relative positions of glycine and alanine in **3**, which are reversed relative to the isomeric **2**. We note that the ^{1}H-nmr signal for H-4′ was again a broad triplet as in **1**, as expected on the introduction in **3** of the alanyl residue in the side-chain at C-5′.

Cycloanchinopeptolide C [**5**] showed a pseudomolecular ion at *m/z* 859 $[M+H+CF_3SO_3H]^+$ in the fabms spectrum in the presence of TMS, corresponding to a mol wt of 708 daltons, as **2** and **3**. Its ^{1}H-nmr spectra (Tables 1 and 2) showed signals for two *p*-hydroxyphenyl residues, one alanyl and one glycyl residue, and the -CHCH$_2$CH$_2$-NH-C(NH$_2$)=NH and -CH$_2$CH$_2$CH$_2$-NH-C(NH$_2$)=NH groupings, with the latter confirmed by COSY nmr. The olefinic signals described in the spectra of anchinopeptolides were missing in **5** and replaced by signals in CD$_3$OD at δ 3.89 (dd, J=10.5 and 4.1 Hz, 10- or 10′-H) coupled with a double doublet at δ 4.80 (J=7.2 and 4.1 Hz, 9- or 9′-H) and at δ 4.06 (dd, J=10.5 and 3.0 Hz, 10′- or 10-H) coupled with a double doublet at δ 4.76 (J=7.2 and 3.0 Hz, 9′- or 9-H). In addition, the signals at δ 3.89 and 4.06 (10- and 10′-H or vice versa) and those at δ 4.80 and 4.76 (9- and 9′-H or vice versa) were coupled each to the other, respectively. These data immediately suggested **5** to be related to anchinopeptolide B [**2**] or C [**3**] by an intramolecular [2+2] cyclo-addition reaction. The ^{1}H-nmr spectrum in DMSO-d_6 showed the alanyl methine proton signal as a quintet at δ 4.42 coupled with an amide proton doublet at δ 8.67 and with the methyl doublet at δ 1.26, thus establishing the alanyl nitrogen to be secondary and accordingly the glycyl nitrogen tertiary as in anchinopeptolide C [**3**]. NOes (nOe difference experiments) were observed between the NH proton resonating at δ 7.32 and the glycyl α-methylene protons at δ 3.85 (m) and between the NH proton resonating at δ 7.91 and the alanyl methine proton at δ 4.42, thus allowing us to distinguish between N_CH and $N_{C'}H$. Then a 2D COSY experiment, which showed cross-peaks between N_CH/H-9 and $N_{C'}H$/H-9′, led to a distinction between the sequences N_CH-C(9)H-C(10)H and $N_{C'}H$-C(9′)H-C(10′)H. NOes observed between N_CH/H-10, $N_{C'}$-H/H-10′, H-9/H-12, H-16, and H-9′/H-12′, H-16′ established the trans relationship of each *p*-hydroxyphenyl group at C-10 and C-10′ with the corresponding side-chain at C-9 and C-9′, respectively. Coupling constants observed for the cyclobutane protons in CD$_3$OD, large for H-9/H-9′ (J=10.5 Hz) and H-10/H-10′ (J=7.2 Hz) when compared with the smallest ones observed for the trans-arranged protons at C-9/C-10 and at C-9′/C-10′ (3.0 and 4.1 Hz or vice versa), were suggestive of a cis relationship between H-9/H-9′ and H-10/H-10′ (4). The relative stereochemistry around the 2-pyrrolidone ring could be confirmed by nOe difference experiments which showed nOe effects at 5-OH/5′-OH, 5-OH/H$_2$-3′, and 5′-OH/H$_2$-3′. Thus, cycloanchinopeptolide C [**5**] is related to anchinopeptolide C [**3**] by an intramolecular head-to-head [2+2] cyclo-addition reaction. Other examples of marine natural products with a cyclobutane ring in their structures formally derived by a [2+2] cyclo-addition reaction are represented by sceptrin, which is a symmetrical dimer of 2-debromooroidin [=hymenidin (5)], from the sponge *Agelas oroides* (6), and the related oxysceptrin, a potent actomyosin activator from *Agelas* cf. *nemoechinata* (7) and *A. coniferin*, debromosceptrin, dibromosceptrin, and debromooxysceptrin (8).

Anchinopeptolides A–D [**1–4**] and cycloanchinopeptolide C [**5**] are new molecular entities that might originate biogenetically by dimerization of two halves, each containing 2-oxo-5-guanidinopentanoyl, L-alanyl (or glycyl), and *p*-hydroxystyrylamino residues, as in Figure 2.

The anchinopeptolides were submitted to a series of binding assays by the screening department of Rhône-Poulenc Rorer and anchinopeptolides B–D [**2–4**] were found to significantly displace specific ligands from the following receptors at 5 μg/ml concentration: somatostatin receptor (from 62% of **3** to 77% of **4**), human B2 bradykinin receptor (from 52% of **3** to 71% of **2**), and neuropeptide Y receptor (from 57% of **3** to 80% of **2**). Anchinopeptolide A [**1**] exhibited a weaker activity (average inhibition values roughly 35–40%) in these receptor binding assays.

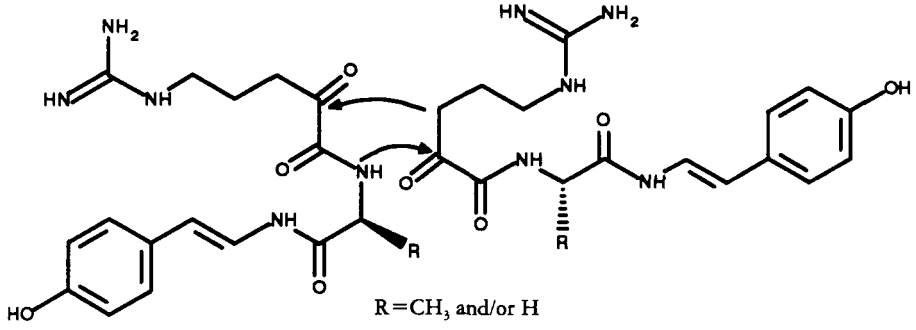


FIGURE 2. Possible Biogenetic Formation of the Five-Membered Ring of Anchinopeptolides A–D [**1-4**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. The 2D COSY, NOESY, and HETCOR experiments were performed by employing conventional pulse sequences. The 2D COLOC experiment was performed according to Kessler (9). Fabms were obtained on a VG ZAB mass spectrometer equipped with a fab source (in glycerol matrix; Xe atoms of energy of 2–6 keV). Optical rotations were determined using a Perkin-Elmer 141 polarimeter, with an Na lamp operating at 589 nm. Uv spectra were from a Beckman DU70 spectrometer. Hplc was performed with a Waters Model 510 pump with refractive index detection.

EXTRACTION AND ISOLATION.—The sponge *Anchinoe tenacior* was collected along the coasts of Tunisia in June 1991 and identified by Professor M. Sarà (Zoological Institute, University of Genova). A zoological sample is kept at Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, under the reference number TU9129. The animals (1.5 kg) were extracted (3×) with Me_2CO at room temperature for two days. The combined extracts (3 liters) were concentrated under reduced pressure, and the remaining aqueous solution was extracted successively with Et_2O and with *n*-BuOH (1.5 liters each). The *n*-BuOH solution was evaporated to dryness to give a residue (4.3 g) which was purified by sequential application of Sephadex LH-20 (eluent MeOH), droplet counter-current chromatography (*n*-BuOH-Me_2CO-H_2O, 3:1:5, ascending mode, the lower phase was used as stationary phase), and hplc [Waters μ-Bondapak C_{18} column (30 cm×3.9 mm i.d.); eluent MeOH-H_2O, 7.5:92.5].

Anchinopeptolide B [**2**].—$[\alpha]_D$ −12.4° (*c*=0.7, MeOH); positive-ion fabms *m/z* $[M+H+CF_3SO_3H]^+$ 859; uv λ max (MeOH) 284 (ε 12070), 318 (sh) nm.

Anchinopeptolide C [**3**].—$[\alpha]_D$ −6.3° (*c*=1, MeOH); positive-ion fabms *m/z* $[M+H+CF_3SO_3H]^+$ 859; uv λ max (MeOH) 284 (ε 14490), 320 (sh) nm.

Anchinopeptolide D [**4**].—$[\alpha]_D$ +11.4° (*c*=1, MeOH); positive-ion fabms *m/z* $[M+H+CF_3SO_3H]^+$ 845; uv λ max (MeOH) 284 (ε 16940), 318 (sh) nm.

Cycloanchinopeptolide C [**5**].—$[\alpha]_D$ +18.6° (*c*=0.5, MeOH); positive-ion fabms *m/z* $[M+H+CF_3SO_3H]^+$ 859; uv λ max (MeOH) 215 (ε 21000), 282 (ε 8080) nm; cd (MeOH) Φ_{280}=0.36, Φ_{215}=1.12.

RECEPTOR BINDING STUDIES.—The in vitro receptor binding assays were performed with membrane preparations from animal tissues (rats or guinea pigs) or cell lines (cells expressing a human gene), according to established methods. The membrane preparations were incubated in optimal conditions of pH, temperature, time, and media, in the presence of the specific radiolabeled ligand with a given concentration of the studied compounds or with a compound possessing a high affinity for the corresponding binding site (non-specific binding measurement). After equilibrium, the mixtures were filtered through a glass fiber filter and the radioactivity remaining in the filter was measured with a scintillator counter.

In particular, the somatostatin and NPY binding assays were performed with membranes from rat celebral cortices as source of receptors and respectively [^{125}I]-somatostatin and [^{3}H]-NPY as radiolabeled ligands. The bradykinin binding assay was performed with the membranes from SF21 cells infected by baculovirus expressing B2 bradykinin receptors and [^{3}H]-bradykinin.

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