Araplysillins-I and -II: Biologically active dibromotyrosine derivatives from the sponge *Psammaplysilla arabica*

A. Longeon[a], M. Guyot[a] and J. Vacelet[b]

[a] *Laboratoire de Chimie, associé au CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, F-75005 Paris (France), and* [b] *Centre d'Océanologie de Marseille, Station Marine d'Endoume, rue de la Batterie des Lions, F-13007 Marseille (France)*

Received 13 March 1989; accepted 11 October 1989

Summary. Araplysillins-I and -II, two novel dibromotyrosine derivatives, were isolated from *Psammaplysilla arabica* and their structure was elucidated by spectroscopic methods. They proved to be inhibitors of Na^+/K^+ ATPase and to have antimicrobial activity.

Key words. Psammaplysilla arabica; dibromotyrosine derivatives; Na^+/K^+ ATPase inhibitors.

In the course of a program devoted to the search for biologically active compounds in marine invertebrates, our attention was focused on the sponge *Psammaplysilla arabica* Keller, 1889 (order Verongida). This sponge was collected in the Red Sea near Djibouti[1] by scuba diving, and the extract (chloroform-methanol 1:1 – fresh sample) was found to exhibit marked antimicrobial activity against *S. aureus* and *E. coli* (Ø inhibition: respectively 23 and 15 mm/mg/disk). Then we carried out a chemical and biological study of this sponge by fractionating the extract and isolating pure compounds, monitoring the process by antimicrobial bioassay.

A first fractionation on a silicagel column, using chloroform with increasing amounts of acetone as eluent, allowed the isolation of several active fractions, eluted respectively with 5%, 10% and 100% acetone; further purification led to isolation of three pure antimicrobial compounds.

The most active component, eluted with chloroform-acetone 10% and purified by preparative TLC (hexane-AcOEt 1:1), m.p. 121–123 °C, M^+ 337, 339, 341, IR: $\nu_{C\equiv N}$ 2260 cm^{-1}, was found to be identical with the previously described and widely distributed aeroplysinin **1** (0.01% dry weight)[2–4]. Aeroplysinin was mainly responsible for the antimicrobial activity of *Psammaplysilla arabica*.

A more polar antimicrobial compound, araplysillin-I, **2a** (0.018% dry weight) was obtained from the fraction eluted with acetone and was purified by chromatography on a column of silanized silica gel 60 (70–230 mesh), eluted with chloroform-methanol 9:1, or preparative TLC on SiO_2 (chloroform-methanol 1:1). Araplysillin-I is a white solid, m.p. 140–142 °C, $[\alpha]_D$: −70° (c = 0.7, methanol), UV: λ_{max}: 283 nm (ε 9392). FAB/MS or araplysillin-I revealed the molecular ions: $[M + H]^+$ 714, 716, 718, 720, 722 in the ratio 1, 4, 6, 4, 1 indicating the presence of four bromine atoms in the molecule. The ^1H NMR (table 1) showed the following signals: δ 6.34 (ethylenic proton), 4.12 (*H*-C-OH), 3.71 (OMe) and an AB system (δ3.80 and 3.03, 1H each, J = 18.3 Hz) characteristic of a spirocyclooxazoline ring system previously encountered in other Verongida constituents: aerothionin **4**[5], fistularin[6] and purealin **5**[7]. The other signals: two aromatic protons, and ten aliphatic protons correlated by decoupling experiments (observed correlations are indicated by square brackets in table 1), suggested the partial structures:

-O-CH_2-CH_2-CH_2-NH-(C = O)- and Ar-CH_2-CH_2-NH_2.

Araplysillin-I, treated with $(Ac)_2O$-Pyr, led to the acetyl acetamide, **2b**. In ^1H NMR of **2b**, we observed a downfield shift for the H-1 (δ 4.12 to δ 5.84) and the CH_2-18 (δ 2.95 to δ 3.47) signals as well as the coupling of the δ 3.47 signal with the NH-Ac, confirming the partial structures proposed above.

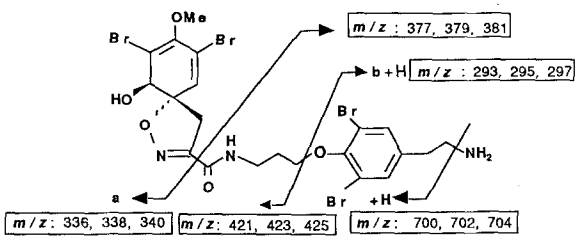
In addition, analysis of the fragmentation in the mass spectra (FAB, scheme 1)[8] and HRMS of fragments **a** and **b**[8] led to structure **2a** for araplysillin-I.

The structure was supported by ^{13}C NMR resonances (table 2), which confirmed the presence of the spirooxazoline ring by comparison with the data described for aerothionin **4**[5,13] and purealin **5**[7].

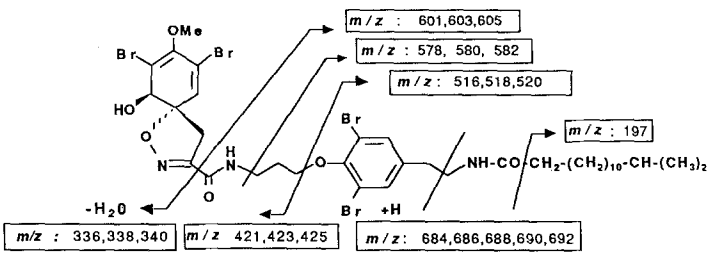
From a less polar fraction eluted with chloroform-acetone (95:5), after preparative TLC (SiO_2; hexane-acetone 95:5), we isolated araplysillin-II **3** (0.003% dry

Table 1. ^1H NMR of **2a**, **2b**, **3** (250 MHz)

	2a(CD_3OD)	**2b**($CDCl_3$)	**3**(CD_3OD)
H-1	4.12 s	5.84 s	4.08 s
H-5	6.34 s	6.36 s	6.41 s
H-7	3.80 d; 3.03 d	3.78 d; 3.08 d	3.78 d; 3.08
	AB syst J = 18.3	AB syst J = 18.3	AB syst J = 18.3
H-10	3.59 t	3.70 dt	3.58 t
H-11	2.10 tt	2.12 tt	2.12 tt
H-12	4.06 t	4.05 t	4.04 t
H-15,15'	7.43 s	7.34 s	7.44 s
H-17	2.74 t	2.73 t	2.73 t
H-18	2.95 bt	3.47 dt	3.37 dt
NH-10	7.33 t	7.33 t	7.31 t
NH-18		5.60 t	5.67 t
H-19			2.12 m
H-20-30			1.25 s
H-31			1.54 m
$(Me)_2$,			0.87 d
OMe	3.71 s	3.76 s	3.72 s
OAc		1.97 s	
NAc		2.14 s	



Scheme 1. Mass fragmentation of araplysillin-I **2a**.



Scheme 2. Mass fragmentation of araplysillin-II **3**.

Table 2. ^{13}C NMR data for **2a**, aerothionin **4** and purealin **5**

	2a[a]	4[b]	5[c]
C-9	159.9 s		
C-8	154.2 s		
C-13	151.6 s		150.7
C-3	148.1 s	149.9	147.0
C-16	137.0 s		
C15,15'	133.3 d		
C-5	130.9 d	130.6	131.1
C-2	121.9 s	121.7	120.7
C14,14'	118.5 s		
C-4	113.5 s	107.8	113.0
C-6	91.9 s	89.7	90.2
C-1	74.1 d	73.2	73.4
C-12	71.3 t		
C-18	42.2 t		
C-7	39.3 t		
C-10	37.3 t		
C-17	35.9 t		
C-11	29.5 t		
OMe	60.2 q		

[a] $CDCl_3$-CD_3OD; [b] $CDCl_3$; [c] DMSO-*d6*.

weight), m.p. 40–42 °C, $[\alpha]_D$: −38° (c = 0.73 chloroform); UV: λ_{max} 270 nm (ε 11 650). The FAB mass spectrum indicated a molecular ion: $[M + H]^+$ 938, 940, 942, 944, 946 (ratio 1, 4, 6, 4, 1). In ^1H NMR (table 1) araplysillin-II exhibited the same characteristic features as **2a** except for one additional amide proton (δ 5.67), a doublet (δ 0.87, 6H) assigned to two terminal methyl groups and the presence of a long straight chain (δ 1.25, 20H). Comparison with araplysillin-I acetyl acetamide **2b**, and fragmentation in mass spectrometry (FAB), led to structure **3** for araplysillin-II (scheme 2).

Like some other dibromotyrosine derivatives, araplysillins-I and-II displayed modest antimicrobial activity: ∅ inhibition 12 mm for **2a** and 7 mm for **3**, 250 µg/disk) against *S. aureus*. More interestingly, they inhibited the activity of purified porcine brain Na^+/K^+/ATPase: ID_{50}: $5 \cdot 10^{-4}$ M for araplysillin-I and 10^{-3} M for araplysillin-II (following the procedure described[9]), more significantly than the parent compounds purealin **5** and lipopurealin[10].

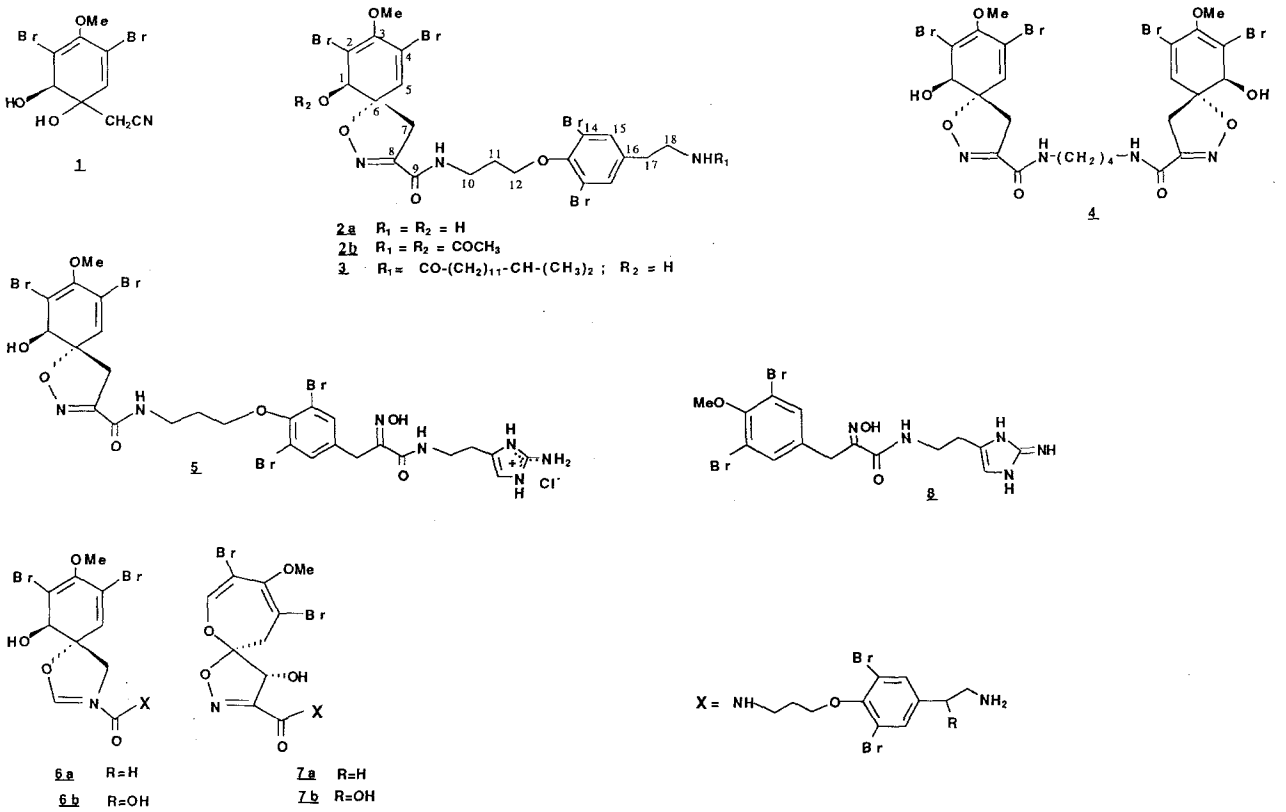
In the family Aplysinellidae, the genus *Psammaplysilla* is well characterized by its skeletal structure[11]. However, diagnostic morphological features have proved difficult to find within the genus. In the Indo-Pacific area, many species have been described under diverse generic names, and have been considered[12] conspecific with *P. purpurea*. A possible exception is *P. arabica*, localized in the Red Sea, to which the present material is tentatively referred. However, to prove this, further studies based on fresh material and field observations are needed.

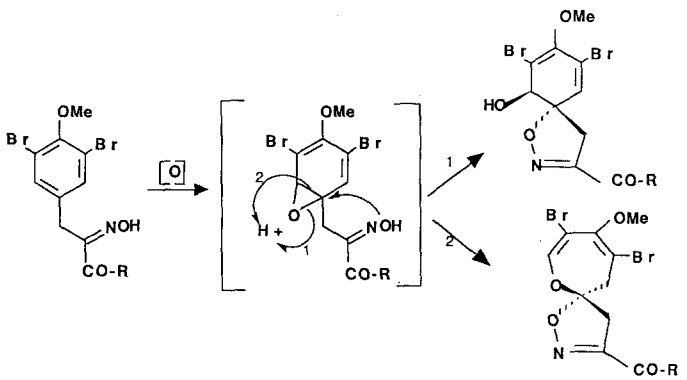
The studies that have been conducted on the bromotyrosine derivatives of this genus demonstrate a certain variability. Kashman's group described the isolation of psammaplysillins-A and -B **6** from specimens collected in the Red Sea (Eilat)[13]. These metabolites were again found by Scheuer's group[14] in specimens collected at Palau in the Pacific, and their structures were revised as **7**. Structure **7** exhibited an original dihydrooxepine moiety never encountered before in Verongidae metabolites, and ^{13}C NMR data, as well as mass spectra, preclude the identity of psammaplysillin-A and araplysillin-I. Moreover, in spite of extensive investigations, we found no compound corresponding to psammaplysillin-B (OH in 17). Hence, from chemical evidence, the specimens studied by Kashman appear to be related to those of Palau, but were different from ours, although both were collected in the Red Sea area.

On the other hand, from a sample collected in the Okinawan Sea and named '*Psammaplysilla purea*', a Japanese group achieved the isolation of purealin **5**[7], which also possesses the spirocyclic oxazoline ring, as well as lipo-purealin[10] which contains an acyl chain instead of the spirooxazoline moiety[7]. These rather confusing data suggest that there might be a common precursor for these metabolites, according to the previously proposed hypothesis[14,15]: aromatic oxidation of an oxime **a** to an epoxide **b** which rearranges either to a spirocyclic oxazoline ring (araplysillin and purealin) or to a dihydrooxepine (psammaplysillins) (scheme 3).

These different processes may depend on the enzyme-pool of the sponge. In fact, metabolites containing such an oxime structure have been described: psammaplin-A (a monobromotyrosine derivative) in a *Psammaplysilla* sp.[16] and ianthellin **8** in another member of the Aplysinellidae family: *Ianthella ardis* (= *Pseudoceratina crassa*)[4]. A biomimetic synthesis was recently carried out[15] which, although proceeding with the very low yields typical of arene oxidations, corroborates this hypothesis.

Whether these conflicting results in *Psammaplysilla* are related to biochemical variability in a single species, or to the existence of a group of related species in the genus, cannot yet be determined.





Scheme 3. Biosynthetic pathway according to Okamoto and Clardy[15].

We thank M. T. Martin for NMR spectra, J. Mercier for HRMS and PIRMED-CNRS for financial support. FAB mass spectra was obtained from the Centre d'Analyse, CNRS, Lyon.

1 *Psammaplysilla arabica* was collected during the Ardoukoba expedition in January 1985.
2 Fulmor, W., Van Lear, G. E., Morton, G. O., and Mills, R. D., Tetrahedron Lett. *1970*, 4551.
3 Fattorusso, E., Minale, L., and Sodano, G., Chem. Commun. *1970*, 751.
4 Litaudon, M., and Guyot, M., Tetrahedron Lett. *27* (1986) 4455.
5 Fattorusso, E., Minale, L., Sodano, G., Moody, K., and Thomson, R. H., Chem. Commun. *1970*, 752.
6 Gopichand, Y., and Schmitz, F. J., Tetrahedron Lett. *1979*, 3921.
7 Nakamura, H., Wu, H., Kobayashi, J., Nakamura, Y., Ohizumi, Y., and Hirata, Y., Tetrahedron Lett. *26* (1985) 4517.
8 Found: 305.859, calculated for $C_8H_4NO_2Br_2$: 305.8549 (a-MeOH:); found: 277.876, calculated for $C_8H_6OBr_2$: 277.876 (294-NH_2)
9 Hosie, R. J. A., Biochem. J. *96* (1965) 404.
10 Wu, H., Nakamura, H., Kobayashi, J., Ohizumi, Y., and Hirata, Y., Experientia *42* (1986) 855.
11 Bergquist, P. R., Pacific Sci. *19* (1965) 123.
12 Bergquist, P. R., New Zealand J. Zool. *7* (1980) 443.
13 Rotem, M., Carmely, S., Kashman, Y., and Loya, Y., Tetrahedron *39* (1983) 667.
14 Roll, D. M., Wang, C. W. J., Scheuer, P. J., Gray, G. A., Shoolery, J. N., Matsumoto, G. K., Van Duyne, G. D., and Clardy, J., J. Am. chem. Soc. *107* (1985) 2916.
15 Okamoto, K. T., and Clardy, J., Tetrahedron Lett. *28* (1987) 4969.
16 Quiñoa, E., and Crews, P., Tetrahedron Lett. *28* (1987) 3229.

0014-4754/90/050548-03$1.50 + 0.20/0