

Brominated Secondary Compounds from the Marine Sponge *Verongia aerophoba* and the Sponge Feeding Gastropod *Tylodina perversa*

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Analysis of the marine sponge *Verongia aerophoba* from the Canary islands afforded the brominated secondary constituents isofistularin-3, aerophobin-1 and aerophobin-2 which are probably involved in the chemical defense of the sponge. In addition the yellow pigment uranidine and the unusual sterol aplysterol were isolated. The patterns of brominated compounds were almost superimposable when samples of *V. aerophoba* from different islands were compared by HPLC indicating *de novo* synthesis by the sponge or by endosymbiotic microorganisms rather than uptake by filter feeding. The only differences observed between the different samples analyzed were with regard to the total concentrations of brominated compounds which varied from 7.2–12.3% of the dry weight dependent on the different collection sites. The Opisthobranch gastropod *Tylodina perversa* is specialized for feeding on *V. aerophoba*. Chemical analysis of the gastropod revealed the sponge constituents uranidine, isofistularin-3, aerophobin-1 and aerophobin-2 as well as aerothionin, a further brominated compound which is apparently a biotransformation product of the brominated sponge constituents.

Introduction

Over the past 20 years marine secondary products have attracted growing interest due to unique chemical features which, for example, frequently include halogen substituents (very rare in secondary products from terrestrial sources) as well as due to pronounced biological activities which suggest potential value as primary structures for the development of new pharmaceuticals [1, 2]. Sessile marine invertebrates, such as sponges (Porifera), have so far yielded the largest number of bioactive secondary metabolites [2]. Sponges are a primitive group of filter-feeders that comprise some 5000 taxa with the majority of them being restricted to the marine environment [3]. Most secondary metabolites from sponges seem to have evolved as chemical defense against predators (e.g. sponge feeding fishes) or for preventing an overgrowth by fouling organisms which cause a threat especially to filter feeders by plugging the pores [4–6]. Field studies conducted in the Gulf of Mexico as well as

at the Great Barrier Reef (Australia), indicated a correlation between the life habit of sponges and the accumulation of ichthyotoxic secondary compounds. Most of the sponges growing exposed on reefs showed aposematic coloration and were found to be strongly toxic to fishes in force feeding experiments whereas most of the nontoxic or mildly toxic sponges were unexposed (for example in caves) and showed cryptic coloration [7, 8].

Verongia aerophoba Schmidt (syn. *Aplysina aerophoba*) is a sponge characterized by a conspicuous sulphurous yellow coloration and a height of ca. 10–20 cm that is found frequently on hard bottom strata in the Mediterranean [9], as well as around the Canary islands. In the present study we report on the brominated secondary constituents of *V. aerophoba* from the Canary islands with emphasis on their possible origin, as well as on compounds from the Opisthobranch *Tylodina perversa* Gmelin (syn. *T. citrina*) [9] that feeds on *V. aerophoba*.

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Materials and Methods

V. aerophoba and *T. perversa* were collected in September and October 1991 by SCUBA diving or



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by snorkeling during the scientific cruise of the RV "Heincke" to the Canary islands. Freshly collected sponges and gastropodes were directly frozen on board at -20°C until extraction. For bulk extraction followed by isolation of brominated secondary compounds lyophilized tissue was ground and extracted either with acetone or with MeOH. Following evaporation of the solvent the extract was partitioned between ethyl acetate and water to remove NaCl. The ethyl acetate fraction was taken to dryness, redissolved in a mixture of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5 v/v) and subjected to column chromatography on silica gel. Fractions (20 ml) were collected and monitored by TLC on premade silica gel plates (Merck, Darmstadt) using the same solvent system. Secondary compounds were detected by their absorbance under $\text{UV}_{254\text{nm}}$ (**1–4**, Fig. 1) or following spraying with anisaldehyde and heating at 110°C (**5**, Fig. 1). Final purification of the isolated compounds was usually achieved by column chromatography on Sephadex LH-20 with MeOH or with mixtures of $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1:1 v/v) as eluents. Alternatively compounds were purified by column chromatography on reversed phase (C_{18}) lobar-columns (Merck, Darmstadt) with mixtures of MeOH and H_2O (for example 80:20 v/v) as eluents.

^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM-300 or WM-400 spectrometers, respectively. All 1D- or 2D-spectra were obtained using the standard Bruker software. Mass spectra (FAB, glycerol as matrix or EI, 70 eV) were measured on a Finnigan MAT 8430 mass spectrometer.

For HPLC analysis 100 mg of lyophilized sponge or gastropod tissue were exhaustively extracted with acetone. An aliquot of the extracts was diluted with H_2O , centrifuged (10,000 g for 5 min) and injected into a HPLC-system (Pharmacia, LKB, Sweden) coupled to a photodiode-array detector (Waters Millipore GmbH, Eschborn, F.R.G.). The separation was achieved by applying a linear gradient from 100% A (10% MeOH, 90% H_2O adjusted to pH 2 with phosphoric acid) to 100% B (MeOH) in 30 min following an isocratic segment at 100% A during the first 5 min of each run. Routine detection was at 254 nm. The separation column (125 \times 4 mm i.d.) was prefilled with Nova-Pak C-18 (4 μm) (Waters Millipore GmbH, Eschborn, F.R.G.). Quantification was achieved

by the external standard method using previously isolated and purified compounds.

Results and Discussion

Sponges of the order Verongida including *V. aerophoba* are a rich source of brominated secondary metabolites presumably originating from 3,5-dibromotyrosine [10–18]. Extraction of *V. aerophoba* from the Canary islands afforded the known brominated compounds isofistularin-3 (**1**), aerophobin-1 (**2**) and -2 (**3**), as well as the pigment uranidine (**4**) and the sterol aplysterol (**5**) (Fig. 1). All compounds were readily identified from their spectroscopic data and by comparison with published data [10–18]. Further brominated metabolites of smaller molecular weight previously reported from *V. aerophoba* such as aeroplysinine-1 (**6**) or the dienone (**7**) [5] were not detected when freeze dried sponge was extracted either with acetone or MeOH. Preliminary data suggest that the latter compounds are enzymatically formed degradation products originating from isofistularin-3 (**1**) or from the aerophobins (**2** and **3**, Fig. 1) upon cellular breakdown ([19], Teeyapant *et al.* in preparation).

Reversed phase HPLC proved to be an excellent tool for the separation and quantification of the brominated secondary compounds in crude solvent extracts of *V. aerophoba* (Fig. 2). The compound patterns of samples of *V. aerophoba* collected from several of the Canary islands including for example Hierro and Alegranza (Fig. 3) proved to be qualitatively homogenous (Fig. 2, Table I). This chemical similarity is remarkable considering the geographical distance of more than 500 km between the latter two islands (Fig. 3). In addition to the distance, the western Canary islands including Hierro show closer biogeographic affinities to the Caribbean region whereas the species distribution around the eastern islands including Alegranza is closer to the Mediterranean and North African region [20, 21]. Considering the distance, as well as environmental differences, between Hierro and Alegranza the striking chemical similarities of the respective sponge samples argue for a *de novo* synthesis either by the sponge itself or by endosymbiotic microorganisms, whereas filter-feeding as an alternative source of the brominated metabolites seems less plausible by comparison. The only

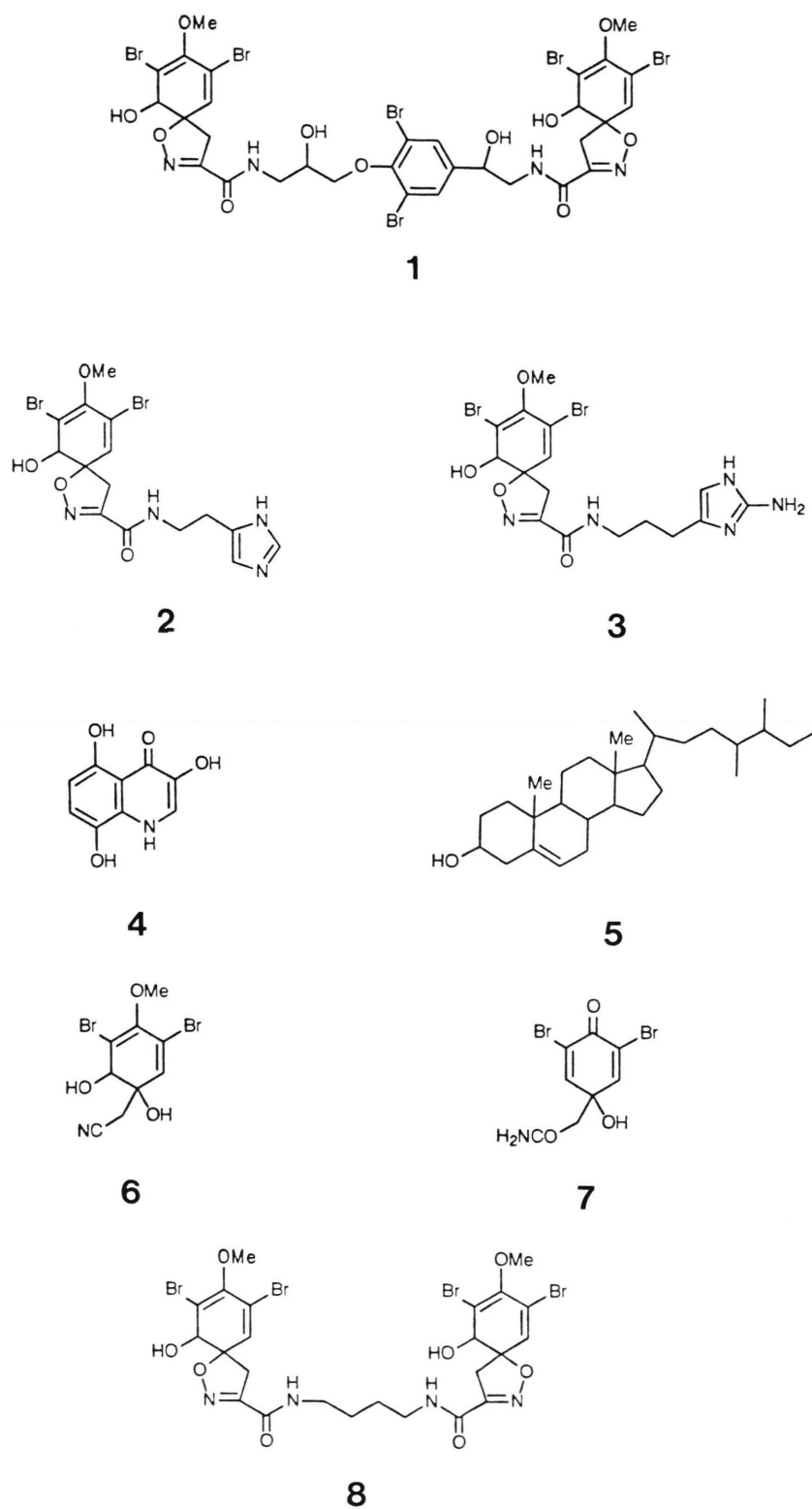


Fig. 1. Secondary constituents from *V. aerophoba* and *T. perversa*.

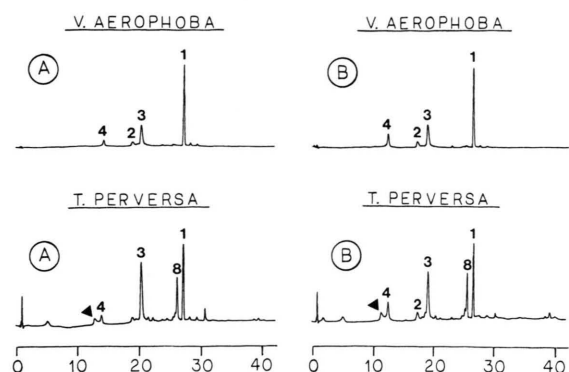


Fig. 2. HPLC analysis of samples of *V. aerophoba* and of *T. perversa* collected at Hierro (A) or at Alegranza (B). Retention times are given in min, peak numbers refer to Fig. 1; ▼ unknown metabolite of uranidine (4).

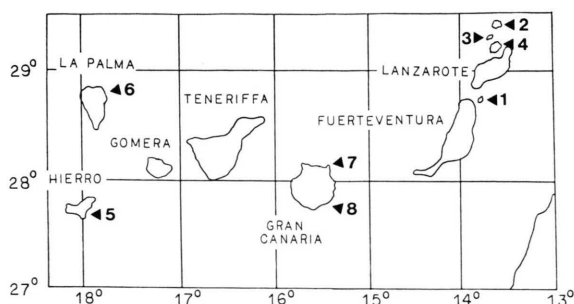


Fig. 3. Map of the Canary islands. Numbers refer to different collection sites:

- | | |
|--------------------|-----------------|
| 1) Lobos | 5) Hierro |
| 2) Alegranza | 6) La Palma |
| 3) Roque del Oeste | 7) Gran Canaria |
| 4) La Graciosa | 8) Gran Canaria |
- Details on the localities can be obtained from the senior author.

differences between the various sponge samples analyzed were with regard to the concentrations of brominated compounds which varied from 7.2–12.3% of the dry weight for the different localities (Table I). Reasons for this quantitative variation are as yet unknown.

V. aerophoba is one of the dominating sponges around the Canary islands in spite of its conspicuous yellow coloration and exposed growing habit which are likely to attract potential sponge feeders. Even though *V. aerophoba* as a member of the Demospongiae lacks protective spicula is apparently well protected as obvious damage attributable to sponge feeders was rarely observed during collection. Furthermore, the sponge surface was usually remarkably free of fouling organisms. A chemical defense of the sponge, possibly mediated by the brominated metabolites (1–3), seems therefore likely. This hypothesis is supported by previous laboratory experiments which demonstrated a pronounced cytotoxic activity of isofistularin-3 (1) [10] that could be ecologically relevant for example in preventing settlement and growth of epibionts. Further studies on the possible defensive role of the sponge secondary metabolites are presently underway.

Even though *V. aerophoba* is apparently well adapted to withstand most potential hazards in its environment the Opisthobranch *Tylodina perversa* Gmelin (syn. *T. citrina*) has been able to overcome the defence mechanism of this sponge. Specimens of *T. perversa* were found associated with half of the sponge samples collected. *T. perversa* usually burrows into the sponge. When present on the sur-

Table I. Abundance of major brominated metabolites in samples of *V. aerophoba* and of *T. perversa* from different collection sites.

Collection site	<i>V. aerophoba</i>			<i>T. perversa</i>			
	Total conc. of brominated compds. in mg/g dry wt.	Abundance in %		Total conc. of brominated compds. in mg/g dry wt.	Abundance in %		
		1	3		1	3	8
1	72.1	42.5	57.5	—	—	—	—
2	123.3	48.6	51.4	41.9	20.9	65.9	13.2
3	122.7	51.7	48.3	—	—	—	—
4	84.5	45.3	54.7	37.6	13.4	73.5	13.1
5	79.7	39.2	60.8	33.6	18.7	61.2	20.1
6	119.2	34.1	65.9	—	—	—	—
7	97.0	35.4	64.6	23.4	31.4	53.0	15.6
8	83.2	29.6	70.4	—	—	—	—

No gastropods were available from collection sites 1, 3, 6 and 8. Numbers of the compounds follow Fig. 1, numbers of the collection sites refer to Fig. 3.

face of the sponge the Opisthobranch is perfectly camouflaged since it exhibits the same sulphurous yellow coloration as *V. aerophoba*. Chemical analysis of *T. perversa* by HPLC with diode-array detection revealed the presence of the sponge pigment uranidine (**4**, Fig. 1) as well as of an unknown metabolite of uranidine that was not present in any of the sponge samples analyzed (Fig. 2). In addition to pigments the brominated sponge constituents isofistularin-3 (**1**), aerophobin-1 (**2**) and -2 (**3**) (Fig. 2) were detected in the gastropod, as well as an additional brominated compound that was identified as arothionin (**8**, Fig. 1) previously isolated from several *Verongia* spp. [14]. Since arothionin (**8**) was lacking in all of the *V. aerophoba* samples analyzed in this study it is assumed that this compound is a metabolite formed by the Opisthobranch from either isofistularin-3 [**1**] or from aerophobin-2 (**3**, Fig. 1). The overall concentrations of brominated metabolites in the different samples of *T. perversa* analyzed varied approximately between 30–45% compared to the concentrations (set at 100%) in the respective sponge samples (Table I). Since the concentra-

tions of brominated compounds in the gastropods appear too large to account for their presence only in the alimentary canal an uptake of **1** or **3** (Fig. 1) from ingested sponge tissues (probably followed by biotransformation yielding arothionin) and subsequent storage in external organs such as mantle or foot seems likely. This assumption is supported by the presence of the sponge pigment uranidine (**4**) in mantle and foot of *T. perversa* causing the same sulphurous yellow coloration of gastropod and sponge.

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- [1] M. H. G. Munro, R. T. Luijbrand, and J. W. Blunt, The Search for Antiviral and Anticancer Compounds from Marine Organisms, in: Bioorganic Marine Chemistry (P. J. Scheuer, ed.), pp. 93, Springer-Verlag, Berlin 1987.
- [2] A. S. Sarma, T. Daum, and W. E. G. Müller, Secondary Metabolites from Marine Sponges, Ullstein Mosby, Berlin 1993.
- [3] R. C. Brusca and G. J. Brusca, Invertebrates, pp. 181, Sinauer Associates, Sunderland 1990.
- [4] J. C. Braekman and D. Daloze, Pure & Appl. Chem. **58**, 357 (1986).
- [5] L. Minale, G. Cimino, S. De Stefano, and G. Sodano, Fortschr. Chemie Org. Naturstoffe **33**, 1 (1976).
- [6] G. J. Bakus, N. M. Targett, and B. Schulte, J. Chem. Ecol. **12**, 951 (1986).
- [7] G. J. Bakus and G. Green, Science **185**, 951 (1974).
- [8] G. Green, Mar. Biol. **40**, 207 (1977).
- [9] R. Riedl, Fauna und Flora des Mittelmeeres, Verlag Paul Parey, Hamburg und Berlin, 3. Auflage (1983).
- [10] G. Cimino, S. De Rosa, S. De Stefano, R. Self, and G. Sodano, Tetrahedron Lett. **24**, 3029 (1983).
- [11] G. Cimino, S. De Rosa, S. De Stefano, A. Spinella, and G. Sodano, Tetrahedron Lett. **25**, 2925 (1984).
- [12] P. De Luca, M. De Rosa, L. Minale, and G. Sodano, J. Chem. Soc. Perkin **1**, 2132 (1972).
- [13] E. Fattorusso, L. Minale, and G. Sodano, Chem. Commun., 751 (1970).
- [14] E. Fattorusso, L. Minale, and G. Sodano, J. Chem. Soc. Perkin **1**, 16 (1972).
- [15] Y. Gopichand and F. J. Schmitz, Tetrahedron Lett. **41**, 3921 (1979).
- [16] A. Kelecom and G. J. Kannengiesser, An. Acad. bras. Cienc. **51**, 633 (1979).
- [17] K. Moody, R. H. Thomson, E. Fattorusso, L. Minale, and G. Sodano, J. Chem. Soc. Perkin **1**, 18 (1972).
- [18] G. M. Sharma, B. Vig, and P. R. Burkholder, J. Org. Chem. **35**, 2823 (1970).
- [19] P. Kreis, R. Teeyapant, S. Zschocke, V. Wray, L. Witte, J. Hacker, and P. Proksch, 40th. Annual Congress on Medicinal Plant Research, Trieste 1992.
- [20] F. Garcia-Talavera, Secr. Publ. Univ. La Laguna, Monografia 10 (1983).
- [21] R. Haroun, W. F. Prud'Homme van Reine, D. G. Müller, E. Serrao and R. Herrera, Helgoländer Meeresunters., in press (1993).