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Chemistry of Two Distinct Aeolid *Spurilla* Species: Ecological Implications

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The lipophilic extracts of two marine aeolid nudibranch molluscs of the genus *Spurilla* collected in distinct geographical areas have been chemically analyzed. The Et_2O extracts of the nudibranchs were dominated by the presence of usual fatty acids and sterols and contained terpenoid compounds **1** – **3** as minor metabolites. Spurillin A (**1**) and spurillin B (**3**) were new molecules whereas *cis*-γ-monocyclofarnesol (**2**) was already reported in the literature as a synthesis product. Interestingly, bursatellin (**4**), previously isolated from anaspidean molluscs of the genus *Bursatella*, was found in the butanol extract of both *Spurilla* species. Compounds **1** – **4** were not detected in the extracts of the sea-anemone preys collected together with the molluscs.

Keywords: Marine natural products, Diterpenes, Bursatellin, Nudibranchs, *Spurilla*.

Introduction

The cladobranch group Aeolidoidea contain about a dozen families constituted by approximately 600 described species.[1 – 3] Members of this taxon are characterized by elongated and tapering bodies, lacking distinct gills and have specialized dorsal appendages, called cerata, that are used in respiration and defense. The cerata contain branches of the digestive gland that transports cnidocysts, which are organelles typically present in cnidarians, to their tips where they are stored in the so-called cnidosacs. Cnidocysts, which consist of minute capsules with an ejectable thread that causes a sting when contacting, are sequestered by the molluscs from various cnidarians food sources, *i.e.* hydrozoans, hexacorals and octocorals, and re-used for their own defense.[4 – 9] The process of incorporation and maintaining cnidocysts in a functional state inside of the mollusc's body is a unique feature in the Aeolidoidea and, with some exceptions, not encountered elsewhere in the molluscs.[2] The mechanism of cnidocysts acquisition from cnidarians has been investigated for some aeolid nudibranchs including *Spurilla* species.[6][8][10] It is assumed that aeolidoideans defend themselves primarily by using these cnidocysts against potential predators.[7] Due to presence of a such main and effective defensive strategy, the use of secondary metabolites as defensive metabolites does not seem to be manifested itself. However, with this regards, chemoecological studies are lacking for aeolid nudibranchs and, more in general, the natural product chemistry of this group of molluscs has been hardly investigated with respect to other opisthobranch taxa.[2][11 – 13] The limited number of chemical data available for Aeolidoidea prevented the definition of a chemical scenario for this group of nudibranchs.[12] Chemicals isolated from aeolid species belong to very different structural classes and include polyhydroxylated steroids,[14] prenylphenols,[15] indole alkaloids,[16][17] carotenoids,[18][19] terpenes.[20 – 23] Such a variety of chemicals are difficult to be rationalised under a chemical marker framework, at least considering the data available until now. Almost compounds isolated from aeolids have a dietary origin even though a possible *de novo* biosynthesis derivation could be considered in some cases. Several members of the Cladobranchia are able to store unicellular algae of the genus *Symbiodinium* (Phylum Dinoflagellata) from their cnidarian food. A few of these species are known to have a high mutualistic relationship with these zooxanthellae, the slugs using the metabolites of the symbionts mainly for their own nutrition.[3][19] A possible defensive role of aeolid secondary metabolites has

been proposed in particular in the genus *Phyllodesmium*[24] which in contrast to almost all other aeolidoideans do not take up cnidocysts from their soft-coral prey, therefore lacking this typical defensive system.[1]

Within the Aeolidiidae, which is one of the largest aeolidoidean family, the genus *Spurilla* Bergh, 1864 has been the object of several taxonomic and biotechnological investigations,[25][26] whereas no chemical data appeared in the literature to date. In the course of our ongoing research on marine molluscs, with the aim at improving the knowledge of the chemistry of scarcely investigated opisthobranch taxa, two distinct *Spurilla* species, the cosmopolitan *Spurilla neapolitana* (Della Chiaje, 1841)[25] from Tyrrenian coasts (Bay of Naples, Italy) and *Spurilla* sp.[27] from Atlantic Ocean (Chubut, Patagonia, Argentina) were studied. The lipophilic extracts of *S. neapolitana* and *Spurilla* sp. were chemically analyzed and compared with those of sea-anemones *Aiptasia diaphana* and *Parabunodactis imperfecta,* on which the animals were observed feeding, respectively. The results of this investigation are here presented.

Results and Discussion

Two lipophilic extracts (Et_2O and butanol soluble portions from the acetone extract) were obtained from each marine organism under investigation. The extracts of the molluscs were carefully analyzed by TLC under various eluent systems and compared with the corresponding extracts of the sea-anemones. The Et_2O extracts of the pair *S. neapolitana* – *A. diaphana* were dominated by the presence of large amounts of usual lipids including fatty acids and sterols and appeared almost comparable. A single spot at R_f 0.25 (petroleum ether/Et_2O, 1:1) was detected exclusively in the extract of the mollusc. This extract was thus purified by silica gel chromatography to give pure compound **1** (see *Experimental Section*). Analogous comparative analysis was conducted on the pair *Spurilla* sp. – *P. imperfecta*. Again, it was observed for both organisms comparable metabolite patterns that were significantly dominated by mixtures of commonly occurring lipids. The extract of the mollusc contained an additional spot at R_f 0.65 (petroleum ether/Et_2O, 1:1) corresponding to a mixture of compounds that was not detected in the sea-anemone extract. The mollusc extract was thus submitted to sequential chromatographic steps including a reversed-phase HPLC purification. Two main peaks corresponding to two pure compounds **2** and **3** were recovered (see *Experimental Section*). The analysis of the butanol extracts of *S. neapolitana* and *Spurilla* sp. showed the presence of a main UV-visible metabolite at R_f 0.5 ($CHCl_3$/MeOH, 8:2) in both molluscs. This metabolite was detected neither in *A. diaphana* nor in *P. imperfecta*. Aliquots of the two mollusc extracts were thus purified by preparative TLC chromatography to give the same compound **4** from two *Spurilla* species (*Fig. 1*).

Preliminary ^{1}H-NMR analysis of the isolated compounds showed that **1** – **3** were unrelated terpenoids all exhibiting hydroxy functionalities whereas **4** was a nitrogen-containing diol. Compounds **1** and **3** were named spurillin A and spurillin B, respectively. Their structure elucidation was conducted as following described and H-atom and carbon NMR values assigned (*Table 1*). Compound **2** was identified as (–)-*cis*-γ-monocyclofarnesol, which, to the best of our knowledge, has never been isolated to date from natural sources whereas it was described as synthesis product.[28 – 30] In particular, the racemic mixture (±)-**2**[28][29] and the (+)-**2** enantiomer[30] were synthesized several years ago and partially characterized. Due to this, fully NMR assignment of compound **2** has been also reported here (*Table 1*). Finally, compound **4** was identified as bursatellin by comparison of ^{1}H- and ^{13}C-NMR, and ESI-MS data with the literature.[31 – 33] Bursatellin was previously isolated from two distinct anaspidean molluscs, *Bursatella leachii pleii*[31] and *Bursatella leachii savignyana*,[32][33] in the two opposite optical forms. The optical rotation values of purified bursatellin (**4**) samples from *S. neapolitana* and *Spurilla* sp. were negative ($[\alpha]_D^{20} = -5$ (c = 0.3, MeOH) and $[\alpha]_D^{20} = -4$ (c = 0.2, MeOH), respectively) revealing that

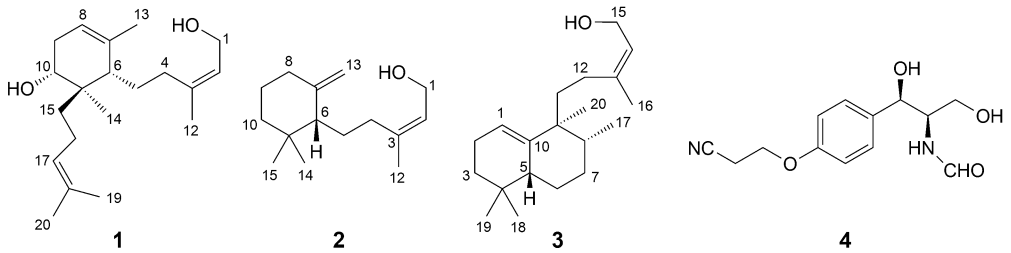

Figure 1. Structures of compounds **1** – **4** from *Spurilla neapolitana* and *Spurilla* sp.

Table 1. ^{1}H- and ^{13}C-NMR data of compounds **1** – **3**, δ in ppm, J in Hz

Position	**1**		**2**		**3**	
	δ(H)[a]	δ(C)[a]	δ(H)[a]	δ(C)[a]	δ(H)[a]	δ(C)[a]
1	4.17 – 4.11 (*m*)	59.1 (*t*)	4.09 (*d*, *J* = 6.9)	59.3 (*t*)	5.36 – 5.32 (*m*)	119.7 (*d*)
2	5.44 – 5.39 (*m*)	123.6 (*d*)	5.40 (br. *t*, *J* = 6.9)	124.1 (*d*)	2.07 – 1.98 (*m*)	23.4 (*t*)
3		140.6[b] (*s*)		140.7 (*s*)	1.39 – 1.33 (*m*)	33.5 (*t*)
					1.17 – 1.12 (*m*)	
4	2.27 – 2.20 (*m*)	34.3 (*t*)	2.01 – 1.97 (*m*)	30.6 (*t*)		32.0 (*s*)
	2.09 – 2.05 (*m*)		1.93 – 1.87 (*m*)			
5	1.78 – 1.74 (*m*)	23.5 (*t*)	1.43 – 1.38 (*m*)	24.9 (*t*)	1.72 – 1.66 (*m*)	43.4 (*d*)
	1.38 – 1.33 (*m*)					
6	1.86 – 1.81 (*m*)	45.1 (*d*)	1.68 (*dd*, *J* = 11.5, 3.6)	53.8 (*d*)	1.60 – 1.56 (*m*)	23.8 (*t*)
					1.29 – 1.25 (*m*)	
7		136.2[b] (*s*)		149.3 (*s*)	1.99 – 1.93 (*m*)	28.5 (*t*)
					1.39 – 1.28 (*m*)	
8	5.26 – 5.21 (*m*)	118.0 (*t*)	2.10 – 2.05 (*m*)	32.8 (*t*)	1.57 – 1.50 (*m*)	38.8 (*d*)
			2.06 – 2.00 (*m*)			
9	2.29 – 2.23 (*m*)	31.9 (*t*)	1.56 – 1.50 (*m*)	23.7 (*t*)		42.5 (*s*)
	2.04 – 1.97 (*m*)					
10	3.69 – 3.62 (*m*)	71.9 (*d*)	1.44 – 1.40 (*m*)	36.6 (*t*)		141.5 (*s*)
			1.25 – 1.19 (*m*)			
11		37.2[b] (*s*)		35.0 (*s*)	1.98 – 1.89 (*m*)	37.8 (*t*)
					1.20 – 1.12 (*m*)	
12	1.77 (*s*)	23.0 (*q*)	1.74 (br. *s*)	23.5 (*q*)	1.92 – 1.88 (*m*)	26.8 (*t*)
					1.74 – 1.66 (*m*)	
13	1.76 (*s*)	23.0 (*q*)	4.80 (*s*)	109.2 (*t*)		141.3 (*s*)
			4.57 (*s*)			
14	0.87 (*s*)	15.8 (*q*)	0.82 (*s*)	25.8 (*q*)	5.37 (br. *t*, *J* = 7.0)	123.3 (*d*)
15	1.44 – 1.40 (*m*)	36.7 (*t*)	0.92 (*s*)	28.5 (*q*)	4.10 (*d*, *J* = 7.0)	59.3 (*t*)
	1.36 – 1.32 (*m*)					
16	2.04 – 1.98 (*m*)	21.8 (*t*)			1.73 (br. *s*)	24.1 (*q*)
	1.96 – 1.92 (*m*)					
17	5.15 – 5.09 (*m*)	124.4 (*d*)			0.81 (*d*, *J* = 7.0)	15.4 (*q*)
18		131.5[b] (*s*)			0.84 (*s*)	25.8 (*q*)
19	1.69 (*s*)	25.5 (*q*)			0.91 (*s*)	28.1 (*q*)
20	1.62 (*s*)	17.3 (*q*)			0.93 (*s*)	21.8 (*q*)

[a] Assignments aided by COSY, HSQC and HMBC experiments. [b] Indirect detection from HSQC and HMBC experiments.

the same laevorotatory isomer[32][33] occurred in both *Spurilla* species.

The molecular formula $C_{20}H_{34}O_2$ of spurillin A (**1**) was established by HR-ESI-MS measurement of the $[M + Na]^+$ ion at *m/z* 329.2471, indicating four indices of hydrogen deficiency. According to a diterpenoid structure, the ^{1}H-NMR spectrum contained five Me *singlets* at δ(H) 0.87 (Me(14)), 1.62 (Me(20)), 1.69 (Me(19)), 1.76 (Me(13)), and 1.77 (Me(12)), that were attributed to one methyl linked to a sp^3 quaternary carbon and four vinyl methyl groups. The ^{1}H-NMR spectrum also showed H-atom *multiplets* at δ(H) 5.15 – 5.09 (H–C(17)), 5.26 – 5.21 (H–C(8)), and 5.44 – 5.39 (H–C(2)) that correlated in the HSQC and HMBC with six olefinic resonances at δ(C) 118.0 (*d*, C(8)), 123.6 (*d*, C(2)), 124.4 (*d*, C(17)), 131.5 (*s*, C(18)), 136.2 (*s*, C(7)), and 140.6 (*s*, C(3)), consistent with three trisubstituted C=C bonds, one of which involved in a terminal dimethylvinyl residue. Signals due to the presence of hydroxy functionalities were observed in the ^{13}C-NMR spectrum at δ(C) 59.1 (*t*, C(1)), and δ(C) 71.9 (*d*, C(10)), as well as in the ^{1}H-NMR spectrum at δ(H) 4.17 – 4.11 (*m*, CH_2(1)) and δ(H) 3.69 – 3.62 (*m*, H–C(10)), according to a primary and a secondary OH groups. The remaining unsaturation degree as required by the molecular formula was thus attributed to a ring. Inspection of 2D-NMR experiments led to the assignment of a cyclohexenol moiety bearing both a pendant prenyl unit and a chain ending with a primary OH function. In particular, COSY and HSQC spectra led us to define the spin systems CH_2(1)/H–C(2), CH_2(4)/H–C(6), H–C(8)/H–C(10), and CH_2(15)/H–C(17) corresponding to four fragments that were assembled by careful analysis of HMBCs. The COSY spectrum showed

cross-peaks of the methylene CH_2(9) (δ(H) 2.29 – 2.23 (*m*) and 2.04 – 1.97 (*m*)) with the vinyl H–C(8) (δ(H) 5.26 – 5.21 (*m*)) and the carbinolic H–C(10) (δ(H) 3.69 – 3.62 (*m*)). The olefinic carbon C(8) was linked to the fully-substituted olefinic C(7) bearing both Me (13) and further connecting methine C(6) as indicated by significant HMBCs between Me(13) (δ(H) 1.76) and C(6) (δ(C) 45.1), C(7) (δ(C) 136.2), and C(8) (δ(C) 118.0). The cyclohexene ring was completed by locating the quaternary carbon C(11) (δ(C) 37.2), which was in turn attached to both Me(14) (δ(C) 15.8) and the prenyl chain, between the methine carbons C(10) (δ(C) 71.9) and C(6) (δ(C) 45.1) by observation of long-range correlations of the singlet at δ(H) 0.87 (Me(14)) with C(6), C(10), C(11), and C(15). In the COSY spectrum, vicinal ^{1}H,^{1}H couplings were also observed between the oxygenated methylene CH_2(1) and the vinylic H–C(2) (δ(H) 5.44 – 5.39), as well as between CH_2(5) (δ(H) 1.78 – 1.74 (*m*) and 1.38 – 1.33 (*m*)) and both CH_2(4) (δ(H) 2.27 – 2.20 (*m*) and 2.09 – 2.05 (*m*)) and H–C(6) (δ(H) 1.86 – 1.81 (*m*)). These two fragments were connected through the fully-substituted sp^2 carbon C(3) (δ(C) 140.6) further bearing Me(12) by diagnostic HMBCs that were observed between the singlet at δ(H) 1.77 (Me(12)) and C(2), C(3), and C(4) thus establishing the OH-containing chain linked to C(6) of the cyclohexene ring. The sequence corresponding to the pendant prenyl unit was finally identified by the COSY coupling of CH_2(16) (δ(H) 2.04 – 1.98 (*m*) and 1.96 – 1.92 (*m*)) with CH_2(15) (δ(H) 1.44 – 1.40 (*m*) and 1.36 – 1.32 (*m*)), and H–C(17) (δ(H) 5.15 – 5.09 (*m*)). This latter H-atom had a direct correlation with the signal at δ(C) 124.4 (C(17)), which showed in turn HMBC cross-peaks with two vinyl methyls at δ(H) 1.62 (br. *s*, Me(20)), and 1.69 (br. *s*, Me(19)), consistent with the terminal dimethylvinyl group. The geometry of the Δ^2 double bond in the chain was established as (*Z*) by the chemical shift of the vinyl Me(12) (δ(C) 23.0) whereas the relative configuration of three stereogenic centers C(6), C(10), and (C11) in the cyclohexene unit was assessed by analysis of NOESY and NOE difference experiments. In particular, diagnostic steric effects were observed between H–C(6) and H–C(10) as well as between Me(14) and CH_2(4) according to the configuration depicted in structure **1** where the OH group and the chain at C(6) were both equatorially oriented and Me(14) was axial, all on the same face of the ring. Consistent with this, the signal due to H–C(10)$_{ax}$ was a broad *multiplet* ($w_{1/2}$ = 17.0 Hz) in the ^{1}H-NMR spectrum whereas axial Me(14) resonated at δ(C) 15.8 in the ^{13}C-NMR spectrum (see *Supporting Information*). This stereochemical assignment was further supported by comparing spectroscopic data of **1** with those reported in the literature for marine diterpenoid models exhibiting a similar structural architecture and substitution pattern[34] (*i.e.* sphaerococcenol A,[35] bromophycolide J[36]). The absolute configuration of spurillin A (**1**) remained undetermined. Unfortunately, every attempt to apply the *Mosher* method failed mainly due to a certain instability observed in the reaction conditions. With the aim at re-isolating additional quantity of spurillin A (**1**) suitable for the planned *Mosher* chemical reaction, further collections of *S. neapolitana* were realized after 2009. The compound was confirmed to occur in all populations analyzed even though the relative concentration of **1** in the extracts was almost variable among different collections. However, very low total amount of spurillin A (**1**) was finally obtained preventing any chemical transformation. Monocyclic diterpenoids with a double pendant prenylation are not common in nature. In particular, in marine organisms, a few examples have been reported from anaspidean molluscs and algae.[34] Biogenetically, they should derive from a cyclization of the open chain C_{20} precursor, initiated specifically at the internal double bond and involving only two C=C bonds.[34]

Compound **3**, named spurillin B, had the molecular formula $C_{20}H_{34}O$ exhibiting two rings and two C=C bonds, as evidenced by analysis of MS and NMR data. The ^{1}H-NMR spectrum of **3** containing five methyl signals at δ(H) 0.81 (*d*, *J* = 7.0, Me(17)), 0.84 (*s*, Me(18)), 0.91 (*s*, Me(19)), 0.93 (*s*, Me(20)), 1.73 (br. *s*, Me(16)), attributed to a vinyl methyl and four methyl groups linked to sp^3 C-atoms, suggested a bicyclic diterpenoid carbon framework. The presence of an allylic hydroxy methylene (HO–C(15)) located on the lateral alkyl chain was indicated by the signal at δ(H) 4.10 (*d*, *J* = 7.0, CH_2(15)), which was coupled to a vicinal olefinic H-atom H–C(14) (δ(H) 5.37, overlapped br. *t*, *J* = 7.0) of a trisubstituted C=C bond. A signal at δ(H) 5.36 – 5.32 (*m*) was assigned to another olefinic H-atom H–C(1) coupled with a methylene (CH_2(2)) resonating at δ(H) 2.07 – 1.98 (*m*), in turn linked to another methylene (CH_2(3), δ(H) 1.39 – 1.33 (*m*) and 1.17 – 1.12 (*m*)). An allylic H-atom H–C(5) at δ(H) 1.72 – 1.66 (*m*) had direct correlation with the signal at δ(C) 43.4 (C(5)), which in turn showed significant long-range cross-peaks with H–C(1) and CH_2(3), in the HMBC spectrum, supporting the location of the second double bond in the ring *A* of a rearranged labdane diterpenoid.[37 – 39] Additional HMBCs were observed between C(5) and the 3 H *singlets* attributed to geminal methyl groups Me(18) and Me(19) confirming the presence of the halimane skeleton.[40] Starting from H–C(5), all carbons and H-atoms of ring *B* were connected by applying a series of 1D- and 2D-NMR

experiments. Particularly diagnostic were the long-range couplings in the HMBC experiment of the quaternary carbon C(9) (δ(C) 42.5) with H–C(8), H_a–C(11), Me (17), and Me(20) as well as C(10) (δ(C) 141.5) with H_a–C(6), and C(1) (δ(C) 119.7) with Me(20). Analogously, all carbons and H-atoms of the lateral alkyl chain C(11)–C(15) were assigned (*Table 1*; *Supporting Information*). The geometry of the double bond in the lateral chain was determined to be (*Z*) the same as in spurillin A (**1**) by the chemical shift of the vinyl Me(16) (δ(C) 24.1). A strong NOE effect between H–C(1) and Me(20) inferred the relative configuration at C(5) and C(9) in agreement with literature models (*i.e.* austrodorin,[41] macfarlandin-C[42]) whereas the configuration at C(8) was suggested the same as in agelasine C[43][44] by the carbon chemical shift value of C(6) (δ(C) 23.8) according to an axial orientation of Me(17) (δ(C) 15.4). Finally, comparison of NMR data of **3** with those of (+)-(5*R*,8*R*,9*S*,13*E*)-halima-1(10),13-dien-15-ol, recently described as an intermediate in the stereospecific synthesis of (+)-agelasine C[44] and differing from **3** only in the geometry of Δ^{13} double bond, confirmed our assignment. Thus, spurillin B (**3**) was assigned as (5*S**,8*S**,9*R**,13*Z*)-halima-1(10),13-dien-15-ol. The absolute configuration remained undetermined.

Conclusions

In summary, the first chemical study of *Spurilla* nudibranchs led to the finding of terpenoid compounds, spurillin A (**1**) from *S. neapolitana*, and (–)-*cis*-γ-monocyclofarnesol (**2**) and spurillin B (**3**) from *Spurilla* sp., whereas bursatellin (**4**),[31 – 33] a nitrogen-containing compound structurally related to chloramphenicol, was isolated from both species.

It is worthy to note that compounds **1** – **4** were not detected in the extracts of cnidarians *A. diaphana* and *P. imperfecta* on which the studied populations of *S. neapolitana* and *Spurilla* sp. were collected, respectively, excluding a dietary origin at least from these two selected sea-anemones. However, it should be considered that the diet of the two nudibranchs includes a certain number of different sea-anemones, in particular *S. neapolitana* has been reported to feed on 37 sea-anemone species.[45] Such a variety makes quite difficult the assessment of alimentary relationships by chemical analysis. On the other side, the trophic relationship of *Spurilla* nudibranchs with hexacorals and in particular the up-take of active cnidocysts from the preys has been demonstrated for both studied species.[6][10][27] Interestingly, it should be remarked that terpenoid compounds are not common metabolites of sea-anemones that are in fact a rich source of proteins and polypeptides,[46] mainly, whereas only a very few reports deals with terpenoids (*i.e.* actiniarins).[47] This should suggest for *Spurilla* terpenoids **1** – **3** either a *de novo* biosynthesis origin or a dietary derivation from different sources including symbiotic microorganisms. Finally, bursatellin (**4**) was detected as main metabolite in both examined *Spurilla* molluscs. This compound was previously reported only from taxonomically unrelated anaspidean *Bursatella* species,[31 – 33] but its origin in the sea hares was not assessed. The finding of **4** also in nudibranchs of the genus *Spurilla* is ecologically relevant and poses intriguing questions about a possible common origin such as dietary zooxanthellae or a *de novo* biosynthesis pathway working in both unrelated genera *Spurilla* and *Bursatella*.

The results obtained by chemical investigation of *Spurilla* genus added new insights in the natural product chemistry of Aeolidoidea nudibranchs. However, further studies on other different *Spurilla* species should be conducted with the aim at establishing if bursatellin could be considered a chemical marker for this genus as well as at verifying the presence of terpenoid metabolites analogous with those occurring in the two species here investigated.

Experimental Section

General Section

Analytical and preparative TLC were performed on precoated SiO_2 plates (*Merck Kieselgel 60 F254*, 0.25 and 0.5 mm), with detection provided by UV light (254 nm) and by spraying with $CeSO_4$ reagent followed by heating (120 °C). SiO_2 column chromatography was performed using *Merck Kieselgel 60* powder (0.063 – 0.200 mm). HPLC separation was carried out using a *Shimadzu LC-10AD* liquid chromatograph equipped with a UV *SPD-10A* wavelength detector (210 nm) on an *RP-C18* semipreparative column (*Kromasil*, 250 × 10 mm, 5 μm, *Supelco*). Optical rotations were measured on a *Jasco DIP 370* digital polarimeter. NMR experiments were recorded at the ICB-NMR Service Centre on a *Bruker DRX 600 MHz* spectrometer equipped with a *TXI CryoProbe* and on a *Bruker Avance-400* spectrometer using an inverse probe fitted with a gradient along the *z*-axis. The NMR spectra were acquired in $CDCl_3$, and the chemical shifts were reported in parts per million referred to $CHCl_3$ (δ 7.26 for H-atom and δ 77.0 for carbon). ESI-MS were performed on a *Micromass Q-TOF MicroTM* coupled with a HPLC *Waters Alliance 2695*. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM,

deviation < 5 ppm RMS in the presence of a known lock mass). Gas chromatography/mass spectrometry (GC/MS): ion-trap mass spectrometer *Polaris Q* (*Thermo*), operating in EI mode (70 eV) connected to a *GCQ* (*Thermo*) gas chromatographer; column: *Trace TR-5* (*Thermo*, 30 m × 0.25 mm × 0.25 μm); helium as gas carrier. High resolution mass spectra (HR-ESI-MS) were acquired on a *Q-Exactive* hybrid quadrupole-orbitrap mass spectrometer (*Thermo Scientific*). GC/MS analysis was carried out on a *Focus GC Polaris Q (Thermo Fisher Scientific*, Waltham, MA, USA).

Animal Material

A population of *S. neapolitana* (28 individuals, average size 3 cm, dry weight 2.0 g) and specimens of anemone *A. diaphana* (90 individuals, dry weight 1.1 g) were collected by scuba diving in Bacoli, Lucrino lake (40°49′52.73″N, 14°5′2.48″E) in March 2009 and in Mar Morto lake (40°49′15″N, 14°4′E) in October 2010, Campi Flegrei, Italy. The animals were identified by one of us (*G. V.*). The biological material was immediately frozen and stored at −20 °C. Specimens of the nudibranch *Spurilla* sp. (34 animals, average size 3 cm, dry weight 3.5 g) were sampled along with the prey, the sea-anemone *P. imperfecta* (10 individuals, dry weight 9.5 g), from rocky intertidal of Larralde Beach (42°24′14″S, 64°18′19W), San José Gulf, (Chubut, Argentina), in January 2009. The animals were identified by one of us (*C. M.*). Immediately after the collection the biological material was frozen and then transferred to ICB laboratories for chemical analysis. Voucher specimens were preserved at Istituto di Chimica Biomolecolare (CNR), Pozzuoli, Naples, Italy (CODE *S. neapolitana*; CODE *A. diaphana*) and at the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN-in: 37723 *Spurilla* sp.; MACN-in: 40975 *P. imperfecta*).

Extraction and Isolation

Frozen individuals of *S. neapolitana* were first immersed in acetone (100 ml × 3) and sonicated with ultrasounds for 1 min. Then, the whole animals were transferred into a mortar, crumbled with a pestle and extracted with acetone threefold. The acetone extracts were combined and the organic solvent was evaporated under reduced pressure to give an aqueous residue. This latter was partitioned in Et_2O (50 ml × 3), and subsequently in BuOH to give 167.1 and 206.5 mg of Et_2O and butanolic extracts, resp. The same extraction procedure was carried out on 90 individuals of *A. diaphana* to afford 113.4 and 139.0 mg of Et_2O and butanolic extracts, resp. The Et_2O extract (167.1 mg) of the mollusc was fractionated on a silica gel column eluted with a gradient of Et_2O in petroleum ether (0 – 100%) and gave ten fractions (*Frs. A – J*), one of which containing the compound of interest (*Fr. I*, 11.3 mg). This fraction was further purified on preparative silica gel TLC plate (petroleum ether/Et_2O, 1:1) to give 1.0 mg of pure spurillin A (**1**). A portion (30 mg) of the butanolic extract of the mollusc was purified on preparative silica gel TLC plate ($CHCl_3$/MeOH, 8:2) to yield 3.0 mg of bursatellin (**4**).

Frozen individuals of *Spurilla* sp. were treated with acetone (100 ml × 3) by using the same procedure as above described for *S. neapolitana*. The aqueous residue from the acetone extract was partitioned in Et_2O (50 ml × 3), and subsequently in BuOH to give 200.0 mg of Et_2O and 265.3 mg of butanol extracts, resp. The same extraction protocol was used for the sea anemone *P. imperfecta* to obtain 650.0 mg and 512.0 mg of Et_2O and butanol extracts. An aliquot (150 mg) of the Et_2O extract of the mollusc was fractionated on a silica gel column eluted with a gradient of Et_2O in petroleum ether (0 – 100%), affording 15 fractions (*Frs. A – O*). The fraction eluted with petroleum ether/Et_2O, 8:2 (*Fr. I*, 9.0 mg) was further purified by reversed-phase HPLC on a *Supelco RP18* column (*Kromasil*, 250 × 10 mm, 5 μm), using an isocratic system (MeCN/H_2O, 9:1, flow rate 1 ml/min) to give (–)-*cis*-γ-monocyclofarnesol (**2**, 1.5 mg, (t_R 13 min),) and spurillin B (**3**, 2.0 mg, t_R 26 min). A portion (30 mg) of BuOH extract of the mollusc was purified by using the same procedure as for the *S. neapolitana* BuOH extract, to afford 2.0 mg of bursatellin (**4**).

Spectroscopic Data of Compounds **1** – **4**

Spurillin A (= **(1*R*,5*S*,6*R*)-5-[(3*Z*)-5-Hydroxy-3-methylpent-3-en-1-yl]-4,6-dimethyl-6-(4-methylpent-3-en-1-yl)cyclohex-3-en-1-ol**; **1**). Colorless oil. $[\alpha]_D^{20} = -4.0$ (c = 0.05, chloroform). ^{1}H. and ^{13}C-NMR: see *Table 1*. LR-ESI-MS: 329.2 ($[M + Na]^+$). HR-ESI-MS: 329.2471 ($[M + Na]^+$, $C_{20}H_{34}NaO_2^+$; calc. 329.2457).

(–)-*cis*-γ-Monocyclofarnesol (= **(2*Z*)-5-[(1*R*)-2,2-Dimethyl-6-methylidenecyclohexyl]-3-methylpent-2-en-1-ol**; **2**). Colorless oil. $[\alpha]_D^{20} = -11.3$ (c = 0.05, chloroform). ^{1}H- and ^{13}C-NMR: see *Table 1*. LR-ESI-MS: 245.2 ($[M + Na]^+$). HR-ESI-MS: 245.1890 ($[M + Na]^+$, $C_{15}H_{26}NaO^+$; calc. 245.1881).

Spurillin B (= **(2*Z*)-3-Methyl-5-[(1*S*,2*R*,4a*R*)-1,2,3,4,4a,5,6,7-octahydron-1,2,5,5-tetramethylaphthalen-1-yl]pent-2-en-1-ol**; **3**). Colorless oil. $[\alpha]_D^{20} = -201.8$ (c = 0.15, chloroform). ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 290.3 (M^+), 191.2 (100), 135.2 (50), 95.1 (40).

Bursatellin (= ***N*-{(1*R*,2*R*)-1-[4-(2-Cyanoethoxy)-phenyl]-1,3-dihydroxypropan-2-yl}formamide**); **4**). $[\alpha]_D^{20} = -5.0$ ($c = 0.3$, methanol) for the sample from *S. neapolitana*; $[\alpha]_D^{20} = -4.0$ ($c = 0.2$, methanol) for the sample from *Spurilla* sp. ^{1}H-NMR (400 MHz, CD_3OD): 7.37 (*d*, *J* = 8.6, H–C(5), H–C(5′)), 6.96 (*d*, *J* = 8.6, H–C(6), H–C(6′)), 4.94 (*d*, *J* = 3.7, H–C(3)), 4.21 (*t*, *J* = 5.7, H–C(8)), 4.16 – 4.11 (*m*, H–C(2)), 3.71 (*dd*, *J* = 11.0, 6.0, H–C(1a)), 3.52 (*dd*, *J* = 11.0, 6.0, H–C(1b)), 2.94 (*t*, *J* = 5.7, H–C(9)). ^{13}C-NMR (100 MHz, CD_3OD): 164.0 (*d*, C(*C*HO)), 158.9 (*s*, C(7)), 136.8 (*s*, C(4)), 128.6 (*d*, C*C*H(5), C*C*H(5′)), 119.2 (*s*, C(*C*N)), 115.4 (*d*, C*C*H(6), C*C*H(6′)), 72.5 (*d*, C*C*H (3)), 64.1 (*t*, C*C*H_2(8)), 62.5 (*t*, C*C*H_2(1)), 57.1 (*d*, C*C*H(2)), 19.0 (*t*, C*C*H_2(9)). LR-ESI-MS: 287.4 ([M + Na]$^+$).

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201700125

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Author Contribution Statement

M. L. C. and *M. C.* performed the chemical and spectroscopic experiments and analyzed the data. *S. G.-M.* and *C. M.* collected, photographed, and identified the biological material from Argentina, and investigated the biological and ecological aspects. *G. V.* contributed the collection and taxonomical studies of Mediterranean animals. *M. R. N.* and *S. G.-M.* made comparative chemical analysis of different populations of the molluscs. *M. L. C.* and *M. G.* conceived and designed the experiments and wrote the paper.

Conflict of Interest

The authors have no conflicts of interest to declare.

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