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Poecillastrosides, Steroidal Saponins from the Mediterranean Deep-Sea Sponge *Poecillastra compressa* (Bowerbank, 1866)

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Abstract: The first chemical investigation of the Mediterranean deep-sea sponge *Poecillastra compressa* (Bowerbank, 1866) led to the identification of seven new steroidal saponins named poecillastrosides A–G (**1**–**7**). All saponins feature an oxidized methyl at C-18 into a primary alcohol or a carboxylic acid. While poecillastrosides A–D (**1**–**4**) all contain an *exo* double bond at C-24 of the side-chain and two osidic residues connected at O-2′, poecillastrosides E–G (**5**–**7**) are characterized by a cyclopropane on the side-chain and a connection at O-3′ between both sugar units. The chemical structures were elucidated through extensive spectroscopic analysis (High-Resolution Mass Spectrometry (HRESIMS), 1D and 2D NMR) and the absolute configurations of the sugar residues were assigned after acidic hydrolysis and cysteine derivatization followed by LC-HRMS analyses. Poecillastrosides D and E, bearing a carboxylic acid at C-18, were shown to exhibit antifungal activity against *Aspergillus fumigatus*.

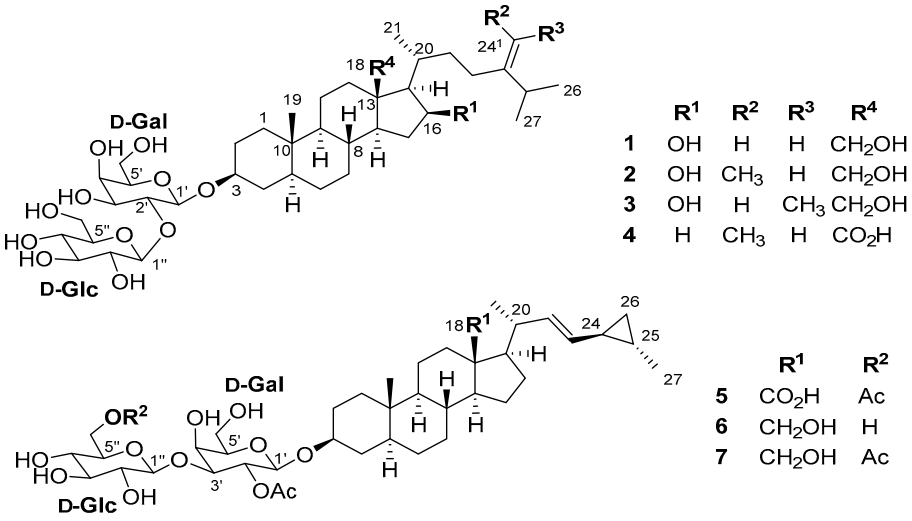
Keywords: sponge; saponins; deep-sea; *Poecillastra compressa*

1. Introduction

In the marine environment, steroid and triterpenoid glycosides are widespread metabolites mainly produced by echinoderms [1–3], although saponins have also been isolated from other marine invertebrates such as octocorals or sponges [4,5]. To date, about 70 saponins have been reported from sponges [6] including sarasinosides from *Asteropus* spp. [7,8], *Melophlus* spp. [9,10], and *Lipastrotethya* sp. [11], ulososides from *Ulosa* sp. [12,13] and *Ectoplyasia ferox* [14], pandarosides

and acanthifoliosides from *Pandaros acanthifolium* [15–18], wondosterols from the association of two sponges [19], erylosides, sokodosides, nobiloside, and formosides from *Erylus* spp. [20–29], ptilosaponosides from *Ptilocaulis spiculifer* [30], mycalosides from *Mycale laxissima* [31,32], feroxosides from *Ectyoplasia ferox* [33], and silenosides from *Silene vulgaris* [34]. While some sponge saponins can be oxidized on the D ring or can contain unusual side chains, the aglycone of most of them belongs to the 30-norlanostane triterpenoid family, with steroidal saponins being rather rare for sponges. Some sponge saponins were subjected to different bioassays and they usually demonstrated interesting biological activities, mostly cytotoxicity against tumor cell lines [35–37].

In our continuous efforts to describe the chemical diversity of marine sponges from the Mediterranean, we undertook the first chemical study of the deep-sea Tetractinellid sponge *Poecillastra compressa* (Bowerbank, 1866). The genus *Poecillastra* is known to produce a broad range of secondary metabolites such as macrolactams [38,39], nitrosohydroxyalkylamines [40], sesquiterpenes, and steroids [41,42]. We report herein the isolation and structure elucidation of seven new steroidal glycosides named poecillastrosides A–G (**1**–**7**) from the deep-sea sponge *P. compressa* (Figure 1). Their structures were deduced from spectroscopic data including 1D- and 2D-NMR experiments as well as high-resolution mass spectra (HRESIMS) analyses. Three different aglycone moieties were identified, and oxidation at the C-18 position is a common feature among all isolated saponins. Poecillastroside A (**1**) contains an ergostane aglycone, whereas poecillastrosides B–D (**2**–**4**) contain a poriferastane, and poecillastrosides E–G (**5**–**7**) a cholestane with a cyclopropyl ring on the side-chain.



	R1	R2	R3	R4
1	OH	H	H	CH_2OH
2	OH	CH_3	H	CH_2OH
3	OH	H	CH_3	CH_2OH
4	H	CH_3	H	CO_2H

	R1	R2
5	CO_2H	Ac
6	CH_2OH	H
7	CH_2OH	Ac

Figure 1. Structure of poecillastrosides A–G (**1**–**7**).

2. Results and Discussion

The freeze-dried sponge sample (43.1 g) was macerated and repeatedly extracted with a mixture of CH_2Cl_2/CH_3OH (1:1) under sonication. The extract (7.9 g) was fractionated by Reversed Phase C18 Vacuum Liquid Chromatography with solvent mixtures of decreasing polarity. The methanolic fraction was then purified by successive RP-Phenylhexyl and C18 HPLC yielding pure compounds **1**–**7**.

Compound **1** was isolated as a yellowish amorphous solid. Its molecular formula $C_{40}H_{68}O_{13}$ was determined by HRESIMS. The ^{1}H NMR spectrum of **1** suggested a steroidal saponin (Table 1). First, the characteristic anomeric signals at δ_H 4.49 (d, J = 7.6 Hz, 1H, H-1′), 4.56 (d, J = 7.9 Hz, 1H, H-1″), and δ_C 101.8 (C-1′), 105.2 (C-1″) evidenced the presence of two sugar residues. The ^{1}H NMR data of the steroid revealed one methyl singlet at δ_H 0.88 (s, 3H, H_3-19), three methyl doublets at δ_H 1.02 (d, J = 6.8 Hz, 3H, H_3-21) and 1.03 (d, J = 6.8 Hz, 6H, H_3-26 and -27), ten methylene groups, an oxygenated methylene with the AB system at δ_H 3.95 and 3.59, a 1,1-disubstituted olefin at δ_H 4.70 and 4.71 (H_2-24^1), seven methine groups, two oxygenated methines at δ_H 3.72 (m, 1H, H-3), 4.26 (td, J = 7.7, 3.7 Hz, 1H, H-16), and three

quaternary carbons at C-10, C-13 and C-24. When compared to usual steroids, this aglycone lacks one characteristic methyl signal for C-18. A hydroxylation was proposed at this position based on the presence of an AB system at δ_H 3.59 (d, *J* = 11.5 Hz, 1H, H-18b) and 3.95 (d, *J* = 11.5 Hz, 1H, H-18a) and further key H-12b, H-14, H-17/C-18, and H_2-18/C-13, C-14, C-17 HMBC correlations. Another unusual feature for the steroid moiety was evidenced in the HSQC spectrum with signals of an oxygenated methine at δ_H 4.26 (td, *J* = 7.7, 3.7 Hz, 1H, H-16) and δ_C 72.8 (CH, C-16). The location of this hydroxyl group at C-16 was confirmed after interpretation of key H-16/H-17 and H-16/H-15a COSY and TOCSY correlations. While most of the relative configurations were in accordance with a common steroid core, the relative configuration at C-16 was established after examination of the NOESY spectrum. Absence of clear nuclear Overhauser effect (nOe) between H-16 and H-14 but also H-18 together with some overlap between H-17 and H-22 did not allow a straightforward determination of the relative configuration at this position. However, H-16/H-15a and H-8/H-15b nOes suggested a β orientation for the hydroxyl group at C-16. As a confirmation of this orientation, the coupling constant values of H-16 were in perfect accordance with those observed for the same signal of a closely related analogue weinbergsterol B, isolated from the sponge *Petrosia weinbergi* [43]. NMR signals of the sugar residues were assigned by extensive COSY, TOCSY, and HSQC interpretation. HMBC experiment evidenced H-5′/C-1′, H-1″/C-2′, H-5″/C-1″ long-range correlations, thus revealing the pyranose nature of these two sugars and their connection at C-2′. Finally, the connectivity of the sugar with the aglycone at C-3 was confirmed through the key HMBC H-1′/C-3 correlation. Moving to the relative configuration of the residues, the large coupling constants between H-1′/H-2′ and H-1″/H-2″ (7.9 and 7.6 Hz, respectively) were consistent with a β configuration for both anomeric centers. This interpretation was confirmed with the one-bond coupling constant $^1J_{CH} \approx 160$ Hz for the two anomeric positions [44]. In addition, the coupling constant values of $^3J_{H3'-H4'}$ 3.2 Hz and $^3J_{H5'-H4'}$ close to zero suggested an axial position for the hydroxyl at C-4 and, therefore, a β-galactopyranosyl residue attached at C-3 of the aglycone [45]. For the second sugar residue, all coupling constants were measured with values between 7 and 9 Hz which implies equatorial positions for all oxygen atoms and, therefore, a β-glucopyranosyl residue connected at C-2′ of the first residue.

Assuming a usual absolute configuration for the aglycone, we turned towards the pyranose moieties. After hydrolysis of the acetal bonds, the resulting monosaccharides were derivatized with L-cysteine methyl ester and phenylisothiocyanate in pyridine [46]. By comparison with standards, a D absolute configuration was assigned for both glucose and galactose monosaccharides.

Compound **2** was isolated as a yellowish amorphous solid. The molecular formula of **2** was determined by HRESIMS as $C_{41}H_{70}O_{13}$. The spectroscopic data were very similar to those of **1**, thereby suggesting that both compounds were close analogues. Examination of the ^{1}H NMR spectrum revealed the presence of an additional methyl group at δ_H 1.59 (d, *J* = 6.3 Hz, 3H, H_3-24^2) placed on the double bond at C-24^1, therefore, leading to a poriferastane skeleton. The relative configuration of **2** was found to be the same as that of poecillastroside A based on nOe correlations. A key H_3-24^2/H_2-23 nOe led us to assign the configuration of the double bond as *E*.

Compound **3** was isolated as a pale yellowish amorphous solid with the same molecular formula $C_{41}H_{70}O_{13}$. Both compounds **2** and **3** are, therefore, isomers. The ^{1}H NMR spectra were almost identical except for a deshielding of the signal corresponding to H-25, from δ_H 2.24 in **2** to δ_H 2.85 for **3**. We first supposed that a change in the configuration of the double had occurred. Due to the low amount of compound available, the corresponding carbons were not visible neither in the ^{13}C NMR spectrum nor in the HSQC, HMBC spectra. We, therefore, decided to enhance the sensitivity of the HSQC spectrum using the recently developed Pure Shift HSQC experiment [47]. Gratifyingly, we were then able to observe both HSQC spots corresponding to C-24^1 and C-25 (Figure S24). The shielding of the C-25 signal from δ_C 36.0 for **2** to δ_C 29.8 for **3** clearly confirmed a *Z* configuration for the double bond of 3.

Table 1. NMR spectroscopic data for poecillastrosides A–D (**1**–**4**) in CD_3OD (500 MHz for ^1H NMR data and 125 MHz for ^{13}C NMR data).

No.	1		2		3		4	
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C
1	1.70, m 0.98, m	38.1	1.69, m 0.98, m	38.1	1.69, m 0.98, m	38.1	1.69, m 0.98, m	38.2
2	1.90, m 1.50, m	30.5	1.90, m 1.50, m	30.5	1.90, m 1.50, m	30.5	1.92, m 1.48, m	30.5
3	3.72, m	80.2	3.72, m	80.2	3.72, m	80.2	3.72, m	80.3
4	1.71, m 1.34, m	35.5	1.71, m 1.34, m	35.6	1.71, m 1.34, m	35.5	1.70, m 1.32, m	35.6
5	1.12, m	46.2	1.12, m	46.2	1.12, m	46.2	1.12, m	46.1
6	1.34, m 1.32, m	29.9	1.34, m 1.31, m	29.9	1.34, m 1.31, m	29.8	1.32, m 1.29, m	29.9
7	1.73, m 0.94, m	33.3	1.74, m 0.94, m	33.3	1.75, m 0.95, m	33.3	1.74, m 0.92, m	33.1
8	1.67, m	36.1	1.67, m	36.1	1.67, m	36.1	1.38, m	38.5
9	0.75, m	56.2	0.74, m	56.2	0.74, m	56.2	0.72, m	55.9
10		36.8		36.9		36.8		36.8
11	1.51, m 1.31, m	22.8	1.52, m 1.32, m	22.8	1.52, m 1.32, m	22.8	1.63, m 1.34, m	24.4
12	2.01, m 1.11, m	38.9	2.01, m 1.10, m	38.8	2.01, m 1.10, m	38.8	2.64, m 1.09, m	38.2
13		48.1		48.1		48.1		55.8
14	1.10, m	55.1	1.10, m	55.1	1.10, m	55.1	1.39, m	58.4
15	2.17, m 1.34, m	38.5	2.16, m 1.33, m	38.6	2.16, m 1.33, m	38.6	1.81, m 1.19, m	26.5
16	4.26, td (7.7, 3.7)	72.8	4.26, td (7.9, 3.7)	72.8	4.26, td (7.9, 3.7)	72.8	1.80, m 0.89, m	24.4
17	1.19, m	62.3	1.19, m	62.3	1.19, m	62.3	1.48, m	57.4
18	3.95, d (11.6) 3.59, d (11.6)	62.6	3.95, d (11.6) 3.60, d (11.6)	62.6	3.95, d (11.6) 3.60, d (11.6)	62.4		180.1
19	0.88, s	12.8	0.88, s	12.8	0.88, s	12.9	0.76, s	12.8
20	1.94, m	31.6	1.93, m	32.2	1.93, m	32.0	1.49, m	38.8
21	1.02, d (6.8)	19.0	1.07, d (6.7)	19.1	1.02, d (6.7)	19.1	1.09, d (6.3)	19.1
22	1.87, m 1.21, m	35.5	1.73, m 1.18, m	35.5	1.83, m 1.18, m	36.8	1.45, m 1.14, m	36.0
23	2.15, m 1.98, m	32.4	2.13, m 1.94, m	26.8	2.04, m 1.83, m	29.1	2.07, m 1.90, m	29.9
24		158.0		148.2		146.9		147.9
24^1	4.71, br s 4.70, br s	106.7	5.19, q (6.7)	116.6	5.17, q (6.7)	117.7	5.18, q (6.7)	116.8
24^2			1.59, d (6.3)	13.4	1.58, d (6.3)	12.8	1.56, d (6.7)	13.4
25	2.29, h (6.5)	34.8	2.24, m	36.0	2.85, m	29.8	2.19, m	35.6
26	1.03, d (6.8)	22.5	0.99, d (6.8)	22.7	0.99, d (6.8)	21.4	0.98, d (6.8)	22.7
27	1.03, d (6.8)	22.3	0.99, d (6.8)	22.6	0.99, d (6.8)	21.4	0.98, d (6.8)	22.6
1′	4.49, d (7.6)	101.8	4.49, d (7.6)	101.8	4.49, d (7.6)	101.8	4.48, d (7.5)	101.8
2′	3.70, m	80.8	3.69, t (10.2)	80.8	3.69, t (10.2)	80.8	3.70, t (10.2)	80.8
3′	3.65, dd (9.6, 3.3)	74.8	3.65, dd (9.6, 3.3)	74.8	3.65, dd (9.6, 3.3)	74.8	3.64, dd (9.5, 3.3)	74.8
4′	3.84, d (3.2)	70.0	3.84, d (3.2)	70.0	3.84, d (3.2)	70.0	3.84, d (3.1)	70.0
5′	3.50, t (6.1)	76.4	3.50, t (6.1)	76.4	3.50, t (6.1)	76.4	3.49, t (6.2)	76.4
6′	3.73, m 3.71, m	62.7	3.73, m 3.71, m	62.7	3.73, m 3.71, m	62.7	3.73, m 3.71, m	62.7
1″	4.56, d (7.9)	105.2	4.56, d (7.9)	105.2	4.56, d (7.9)	105.2	4.56, d (7.9)	105.2
2″	3.25, dd (9.1, 7.9)	75.8	3.25, dd (9.1, 7.9)	75.8	3.25, dd (9.1, 7.9)	75.8	3.25, dd (9.0, 7.8)	75.8
3″	3.37, t (8.8)	77.7	3.37, t (8.8)	77.7	3.37, t (8.8)	77.7	3.37, t (8.9)	77.7
4″	3.33, t (9.3)	71.4	3.33, t (9.3)	71.4	3.33, t (9.3)	71.4	3.33, t (9.4)	71.4
5″	3.29, m	78.4	3.29, m	78.4	3.29, m	78.4	3.28, m	78.4
6″	3.84, dd (11.2, 2.3) 3.71, m	62.4	3.84, dd (11.1, 2.3) 3.71, m	62.4	3.84, dd (11.1, 2.3) 3.71, m	62.4	3.84, dd (13.5, 2.8) 3.71, m	62.4

Compound **4** was isolated as a pale yellowish amorphous solid with a molecular formula $C_{41}H_{68}O_{13}$. The ^1H NMR spectrum of **4** was very similar to the one of **2** except for the absence of the signals corresponding to the AB system of H_2-18 and a shielding observed for δ_H 2.64 (m, 1H, H-12a). The only explanation consistent with all these observations, including the molecular formula, was the replacement of the hydroxyl group at C-18 by a carboxylic acid. This interpretation was further supported by a key H-17/C-18 HMBC correlation. Based on the chemical shift of the signal H-25 the configuration of the double bond was found to be the same as in **2**.

Compound **5** was isolated as a white amorphous solid with a molecular formula of $C_{43}H_{66}O_{15}$. Despite strong differences when compared with **1**–**4**, the NMR data of **5** evidenced that the molecule was a steroidal saponin (Table 2). The aglycone exhibited an unusual skeleton with the presence of a terminal methylated cyclopropyl ring on the lateral chain. This assumption was based on the shielded signals of H-25 and H-26 but also by COSY, HSQC, and HMBC data analyses with the key

H-27/C-24, H-27/C-26 HMBC correlations. Further analysis of ^{1}H NMR data revealed the *E* geometry of the olefinic bond ($J_{H\text{-}22,\text{-}23}$ = 15.2 Hz). No clear nOe correlations were observed for assessing the relative configuration around the cyclopropane ring. Gratifyingly, comparison with literature data and synthetic analogues of sterols with an identical side-chain led us to propose a *trans* configuration for the substituents at C-24 and C-25 of this ring [48–51]. To confirm this configuration in our case, we decided to look further into the coupling constants of the signals corresponding to the cyclopropane protons. Only the signals of the methylene and their multiplicity were clearly identified in the ^{1}H NMR spectrum (Figure 2). In the case of a *trans* configuration of the two substituents around the cyclopropane, H_a and H_b would have the same splitting pattern as they would have in the presence of a pseudo C2 axial symmetry perpendicular to the cyclopropane plane. The ^{3}J coupling constants between protons in a *cis* configuration are known to be between 8 and 10 Hz while values below 7 Hz are always observed when placed in a *trans* configuration. The multiplicity for both signals is observed as a doublet or triplet with coupling constants around 8 and 4 Hz, respectively. This same splitting pattern for both signals is only consistent for a *trans* configuration. Indeed, for a *cis* configuration, one of the two *gem* protons H_b would exhibit two large ^{3}J coupling constants of 8 Hz. We, therefore, confirm a *trans* configuration for the two substituents and estimate the *gem* ^{2}J coupling constants between H_a and H_b to be around 4 Hz. The presence of a carboxyl group at C-18 was inferred first from the HRESIMS data and then from the deshielding of H-12a, exactly in the same manner as for compound **4**. Another difference with **4** arose from the absence of the signal corresponding to the oxygenated methine at C-16. This feature was confirmed by COSY, HSQC, and HMBC correlations. Looking at the glycosidic part of the saponin, the relative configuration was similar to those of **1**–**4**, therefore, confirming one galactose linked to the aglycone and one glucose linked to the galactose. HMBC showed long-range correlations between H-1″/C-3′, H-2′/C_{Ac} (δ_C 172.2), and H-6″/C_{Ac} (δ_C 172.8), thereby indicating the presence of two acetyl groups at C-2′ and C-6″. Unlike compounds **1**–**4**, the glycosidic link between both sugar residues was placed at C-3′ of the galactose. Deshielding of the signal of C-3′ at δ_C 82.4 in the ^{13}C NMR spectrum confirmed this new substitution pattern.

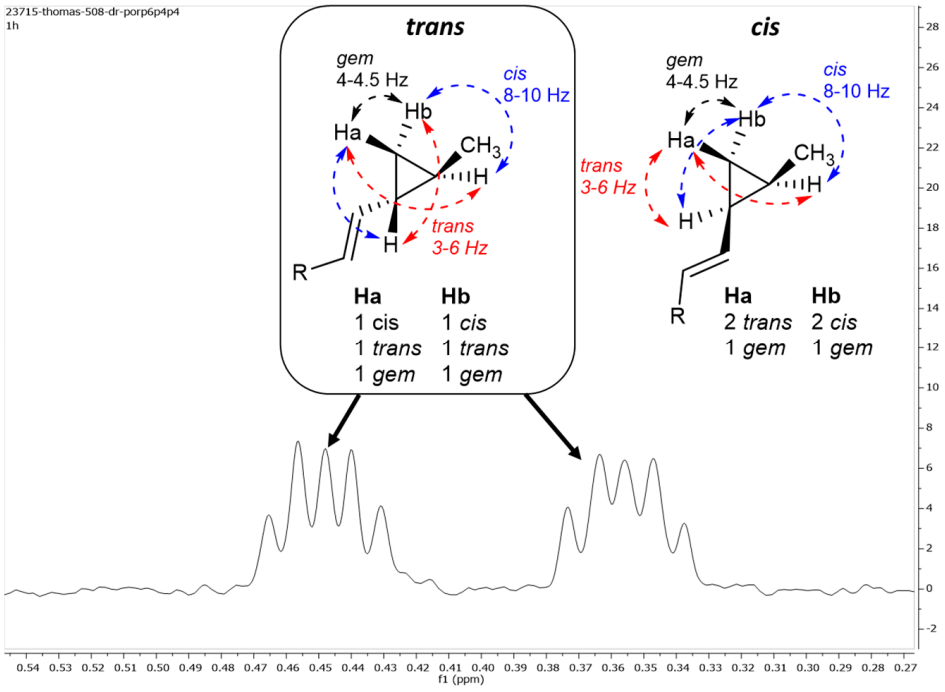


Figure 2. Assignment of the relative configuration of the disubstituted cyclopropane through ^{1}H NMR coupling constants [52].

Table 2. NMR spectroscopic data for poecillastrosides E–G (**5**–**7**) in CD_3OD (500 MHz for ^1H NMR data and 125 MHz for ^{13}C NMR data of **5**; 600 MHz for ^1H data and 150 MHz for ^{13}C data of **6** and **7**).

No.	5		6		7	
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C
1	1.70, m 0.97, m	38.0	1.72, m 0.97, m	38.2	1.72, m 0.98, m	38.2
2	1.85, m 1.44, m	30.4	1.86, m 1.46, m	30.7	1.87, m 1.46, m	30.8
3	3.62, m	79.9	3.63, m	80.0	3.62, m	80.0
4	1.58, m 1.17, m	35.8	1.58, m 1.17, m	35.9	1.58, m 1.19, m	36.0
5	1.12, m	46.0	1.09, m	46.1	1.10, m	46.1
6	1.32, m 1.29, m	30.3	1.32, m 1.29, m	29.9	1.31, m 1.27, m	30.4
7	1.76, m 0.94, m	33.1	1.68, m 0.87, m	33.5	1.67, m 0.87, m	33.5
8	1.53, m	38.8	1.43, m	37.1	1.43, m	37.0
9	0.73, m	55.9	0.68, m	56.0	0.68, m	56.0
10		36.7		36.8		36.8
11	1.63, m 1.31, m	24.4	1.53, m 1.36, m	22.3	1.53, m 1.34, m	22.3
12	2.63, m 1.10, m	38.4	2.44, d (12.8) 0.94, m	35.9	2.44, dt (12.7, 3.4) 0.94, m	35.9
13		55.6		47.9		47.9
14	1.38, m	58.4	1.11, m	57.6	1.12, m	57.6
15	1.75, m 1.30, m	30.8	1.70, m 1.30, m	29.9	1.71, m 1.29, m	29.9
16	1.78, m 1.53, m	25.8	1.54, m 0.98, m	25.0	1.54, m 0.98, m	24.9
17	1.46, m	57.3	1.15, m	58.2	1.16, m	58.1
18		180.1	3.65, d (11.5) 3.45, d (11.6)	60.2	3.65, d (11.1) 3.45, d (11.7)	60.4
19	0.73, s	12.7	0.83, s	12.7	0.83, s	12.7
20	1.92, m	42.4	2.26, m	41.7	2.26, m	41.7
21	1.07, d (6.3)	21.2	1.07, d (5.9)	22.1	1.07, d (6.4)	22.1
22	5.21, dd (15.1, 8.5)	134.6	5.22, dd (14.8, 9.0)	136.0	5.22, dd (15.2, 8.9)	136.0
23	4.90, m	132.4	4.94, dd (14.8, 8.1)	131.6	4.94, dd (15.2, 8.3)	131.6
24	0.96, m	23.4	0.93, m	23.4	0.93, m	23.4
25	0.62, m	15.5	0.62, m	15.5	0.62, m	15.5
26	0.44, td (9.0, 4.5) 0.36, dt (9.0, 4.5)	15.2	0.45, m 0.36, m	15.2	0.45, m 0.35, m	15.2
27	1.03, d (5.9)	18.8	1.03, d (5.8)	18.9	1.03, d (5.9)	18.9
1′	4.56, d (8.0)	101.1	4.55, d (7.9)	101.2	4.56, d (8.0)	101.2
2′	5.11, dd (8.4, 8.1)	72.5	5.12, dd (9.0, 7.7)	72.6	5.11, dd (10.1, 8.0)	72.4
2′-Ac	2.06, s	21.2 172.2	2.06, s	21.2 171.2	2.06, s	21.2 172.2
3′	3.76, dd (10.2, 3.3)	82.4	3.80, dd (10.0, 2.8)	82.2	3.76, dd (10.1, 3.2)	82.4
4′	4.07, d (3.2)	70.2	4.11, d (3.1)	70.2	4.07, d (3.4)	70.2
5′	3.55, t (6.1)	76.4	3.56, t (6.2)	76.4	3.55, t (6.4)	76.4
6′	3.74, m 3.73, m	62.3	3.74, m 3.72, m	62.2	3.74, m 3.72, m	62.1
1″	4.39, d (7.6)	106.0	4.38, d (7.9)	106.0	4.38, d (8.0)	106.0
2″	3.21, t (8.3)	74.6	3.19, t (8.3)	74.8	3.21, t (8.3)	74.7
3″	3.32, t (10.1)	77.7	3.35, m	77.9	3.33, m	77.9
4″	3.28, t (9.6)	71.6	3.28, m	71.3	3.29, m	71.5
5″	3.46, m	75.3	3.64, m	80.0	3.46, m	75.3
6″	4.38, d (11.9) 4.20, dd (11.9, 6.1)	64.7	3.84, m 3.67, m	62.5	4.38, dd (11.9, 2.7) 4.20, dd (11.9, 6.2)	64.7
6″-Ac	2.06, s	20.8 172.8			2.06, s	20.8 172.8

Compound **6** was isolated as a white amorphous solid with a molecular formula of $C_{41}H_{66}O_{13}$. The spectroscopic data were very similar to those of **5**, thereby suggesting a close aglycone moiety. However, some changes were noticed by HSQC and HMBC analyses. Indeed, in the aglycone moiety, we observed the same AB system for H_2-18 as that present in compounds **1**–**3**. The long-range H-17/C-18 HMBC correlation confirmed the presence of an oxygenated methylene at C-13. In the D-β-glucose residue, the chemical shifts, and the COSY data were consistent with a terminal primary alcohol at C-6″, thereby implying the loss of the acetate at this position.

Compound **7** was isolated as a white amorphous solid with a molecular formula $C_{43}H_{68}O_{14}$. The ^1H NMR spectrum evidenced the fact that **7** is a close analogue of **6**. The long-range H-6″/C_{Ac}

(δ_C 172.8) HMBC correlation revealed the presence of an acetate group linked at O-6" as in compound **5**. The relative configuration of **7** was the same as those of **5** and **6**.

Poecillastrosides A–G were tested in a panel of antimicrobial and cytotoxicity assays, including antibacterial activity against Gram positive (methicillin resistant (MRSA) and methicillin sensitive (MSSA) *Staphylococcus aureus*), and Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*), antifungal activity against *Aspergillus fumigatus*, and cytotoxicity against the hepatic tumoral cell line hep_G2. Poecillastrosides D (**4**) (MIC_{90} = 6 μg/mL) and E (**5**) (MIC_{90} = 24 μg/mL) were the only two molecules active in the assay against *A. fumigatus*, revealing a key role of the carboxylic acid functionality at C-18 in the antifungal activity of this structural class. On the other hand, cytotoxicity assays also revealed weak activity of some members of the family against the hep_G2 human cell line, with IC_{50} values of 38, 28, and 89 μg/mL for poecillastrosides B, C, and D (**2**–**4**), respectively. None of the compounds of this family displayed activity against the bacterial pathogens at the highest concentration tested (96 μg/mL for compound **1**–**5**, and 64 μg/mL for compounds **6** and **7**).

3. Material and Methods

3.1. General Experimental Procedures

Optical rotations were recorded with a PerkinElmer 343 polarimeter equipped with a 10 cm microcell and a sodium lamp. UV measurements were obtained by extraction of the Diode Array Detector (DAD) signal of the Ultra-High Pressure Liquid Chromatography (UHPLC) Dionex Ultimate 3000 (Thermo Scientific, Waltham, MA, USA). NMR experiments were performed on a 500 MHz (Advance, Bruker, Billerica, MA, USA) or a 600 MHz (Agilent, Santa Clara, CA, USA) spectrometer. Chemical shifts (δ in ppm) are referenced to the carbon (δ_C 49.0) and residual proton (δ_H 3.31) signals of CD_3OD. High-resolution mass spectra (HRESIMS) were obtained from a mass spectrometer Agilent 6540. HPLC separation and purification were carried out on a Jasco LC-2000 series equipped with a UV detector coupled with an Evaporative Light Scattering Detector, ELSD (Sedere, Alfortville, France).

3.2. Biological Material

Poecillastra compressa (Bowerbank, 1866) was collected in the Mediterranean Sea, off the French coasts, on 15 October 2014 at 200 m depth using a Remotely Operated Vehicle (Super Achille, COMEX S.A., Marseille, France). The voucher specimen "CS2ACHP09_ECH04" is kept at the Marine Station of Endoume (OSU Institut Pythéas, Marseille, France).

3.3. Extraction and Isolation

The dry sponge sample (43.1 g) was ground with a mortar and extracted with a mixture of CH_3OH/CH_2Cl_2 (1:1, *v*/*v*) at room temperature, yielding 7.9 g (18% yield from dry-weight) of extract after solvent evaporation. The crude extract was fractionated by RP-C18 vacuum liquid chromatography (elution with a decreasing polarity gradient of H_2O/CH_3OH from 1:0 to 0:1, then CH_3OH/CH_2Cl_2 from 1:0 to 0:1). The CH_3OH (422 mg) fraction was then subjected to RP-HPLC on a preparative phenylhexyl column, 250 mm × 19 mm, 5 μm (Xselect, Waters, Milford, CT, USA), using a mobile phase of water (A) and acetonitrile (B). The method was developed on 30 min acquisition time: isocratic 60% B for 15 min, then linear gradient to 98% B in 1 min, held at 98% B for 10 min, back to 60% B in 1 min, and held at that percentage of B for 3 min. Selected fractions from this chromatography were then purified by RP-HPLC on a semi-preparative HTec C18 column, 250 mm × 10 mm, 5 μm (Nucleodur, Macherey-Nagel, Düren, Germany), with the following methods for each subsequent purification: isocratic 47% B to afford pure **1** (4.3 mg, $9.98 \times 10^{-3}\%$ *w*/*w*), isocratic 49% B to afford **2** (6.2 mg, $1.44 \times 10^{-2}\%$ *w*/*w*) and **3** (1.4 mg, $3.49 \times 10^{-3}\%$ *w*/*w*), isocratic 50% B to afford **4** (1.6 mg,

3.71 × 10^{-3}% *w*/*w*), isocratic 51% B to afford **5** (0.9 mg, 2.09 × 10^{-3}% *w*/*w*), and isocratic 53% B to afford **6** (0.7 mg, 1.62 × 10^{-3}% *w*/*w*) and **7** (0.8 mg, 1.86 × 10^{-3}% *w*/*w*).

Poecillastroside A (**1**): Yellow, amorphous solid; $[\alpha]_D^{20}$ +12.8 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 195 nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (−) *m*/*z* 755.4582 $[M - H]^-$ (calcd. for $C_{40}H_{67}O_{13}$, 755.4587, Δ − 0.7 ppm).

Poecillastroside B (**2**): Yellow, amorphous solid; $[\alpha]_D^{20}$ +13.2 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 210 nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (−) *m*/*z* 769.4743 $[M - H]^-$ (calcd. for $C_{41}H_{69}O_{13}$, 769.4744, Δ − 0.1 ppm).

Poecillastroside C (**3**): Yellow, amorphous solid; $[\alpha]_D^{20}$ +13.0 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 212 nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (−) *m*/*z* 769.4745 $[M - H]^-$ (calcd. for $C_{41}H_{69}O_{13}$, 769.4744, Δ + 0.1 ppm).

Poecillastroside D (**4**): Yellow, amorphous solid; $[\alpha]_D^{20}$ +8.9 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 222 nm; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (+) *m*/*z* 791.4567 $[M + Na]^+$ (calcd. for $C_{41}H_{68}NaO_{13}$, 791.4563, Δ + 0.5 ppm).

Poecillastroside E (**5**): White, amorphous solid; $[\alpha]_D^{20}$ −6.2 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 220 nm; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS (+) *m*/*z* 845.4307 $[M + Na]^+$ (calcd. for $C_{43}H_{66}NaO_{15}$, 845.4299, Δ + 0.9 ppm).

Poecillastroside F (**6**): White, amorphous solid; $[\alpha]_D^{20}$ −27.3 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 222 nm; ^1H NMR and ^{13}C NMR data, see Table 2; HRESIMS (+) *m*/*z* 789.4405 $[M + Na]^+$ (calcd. for $C_{41}H_{66}NaO_{13}$, 789.4401, Δ + 0.5 ppm).

Poecillastroside G (**7**): White, amorphous solid; $[\alpha]_D^{20}$ −14.1 (*c* 0.1, CH_3OH); UV (DAD) λ_{max} 225 nm; ^1H NMR and ^{13}C NMR NMR data, see Table 2; HRESIMS (+) *m*/*z* 831.4518 $[M + Na]^+$ (calcd. for $C_{43}H6_8NaO_{14}$, 831.4507, Δ + 1.3 ppm).

3.4. Determination of the Absolute Configuration of the Pyranoses

Hydrolysis of glycosides and derivatization of the subsequent monosaccharides were performed individually following previously described methodologies [46]. The monosaccharide derivatives separation was carried out by UHPLC-HRMS on Acquity BEH (Ethylene Bridged Hybrid) C18 1.7 µm, 2.1 mm × 100 mm (Waters). The column was heated at 40 °C. The eluent consisted of water with 0.1% formic acid (A) and acetonitrile/methanol/isopropanol (50:25:25, *v*/*v*/*v*) with 0.1% formic acid (B). The analysis was performed in isocratic mode at 13% B and at a flow rate of 360 µL/min. The injection volume was set at 3 µL. The identity of all monosaccharide derivatives was confirmed after extraction of the ion $[M + H]^+$ at *m*/*z* 433.1098 (Figure S55).

3.5. Evaluation of the Biological Activities

Compounds **1**–**7** were tested for their ability to inhibit the growth of Gram positive bacteria (*S. aureus* ATCC29213 (MSSA), and *S. aureus* MB5393 (MRSA)) and Gram negative bacteria (*E. coli* ATCC25922, *K. pneumoniae* ATCC700603, *P. aeruginosa* PAO1, and *A. baumannii* CL5973), and fungi (*A. fumigatus* ATCC46645), following previously described methodologies [53,54]. Cytotoxic activity against the hepatic human tumoral cell line hep_G2 was determined as previously reported [55].

4. Conclusions

Poecillastrosides A–G (**1**–**7**) share an unusual oxidized methyl at C-18, and they are the first saponins exhibiting this feature. The structures of poecillastrosides E–G (**5**–**7**) also incorporate a

terminal methylated cyclopropyl ring already known in some sponge steroids and already investigated for biosynthetic studies [56]. This cyclopropanation process could lead to the cholestane skeleton, then ergostane, and finally poriferastane, all of them being present in the metabolome of this sponge. Many sterols containing a cyclopropyl ring have been isolated to date [57], but to our best knowledge, this is the first time that saponins containing a 3-membered ring on the side-chain have been reported. Poecillastrosides D (**4**) and E (**5**), bearing a carboxylic acid at C-18, were found to be the most bioactive compounds in the antimicrobial bioassays with an interesting antifungal activity against *Aspergillus fumigatus*.

Supplementary Materials: The following are available online at www.mdpi.com/1660-3397/15/7/199/s1: HRMS and ^{1}H, ^{13}C, COSY, TOCSY, HSQC, HMBC, and NOESY NMR data for compounds **1**–**7** as well as procedures for absolute configuration of compound **3**.

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Conflicts of Interest: The authors declare no conflict of interest.

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