New Antibacterial Diterpenes from *Pseudopterogorgia elisabethae*

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From the MeOH extract of *Pseudopterogorgia elisabethae*, collected from the Bahamas, four new diterpenes, elisabethin E (**1**), elisabethin F (**2**), pseudopterosin P (**3**), and pseudopterosin Q (**4**), were isolated and their structures established with the aid of extensive spectroscopic studies. Compounds **3** and **4** showed antibacterial activity selectively against the *Gram*-positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

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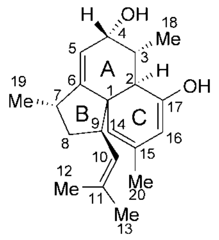
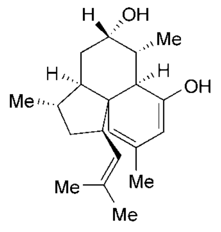
1. Introduction. – New antibiotics are a vital necessity to protect human health. Marine organisms, particularly soft corals, are sources of novel natural products exhibiting various promising bioactivities such as anti-inflammatory, anticancer, antituberculosis, and antibacterial properties [1][2]. Marine organisms have attracted the attention of organic chemists to explore these hidden resources for biomedical applications. *Pseudopterogorgia elisabethae* is one of the soft corals. It is abundant in the Caribbean region and is a rich source of useful biomedical agents. For instance, the most-potent anti-inflammatory agents, the pseudopterosins A – L, were isolated from *P. elisabethae* collected from the Bahamas [3–5]. Novel diterpenes and diterpene alkaloids exhibiting anticancer and antituberculosis activities were isolated from the same species collected from the Florida Keys and San Andres Island, Colombia [6–10].

We initiated a project to isolate natural products exhibiting antibacterial activity from marine soft corals. In our initial screening, the crude MeOH extract of *P. elisabethae* showed strong antibacterial activity. On this basis, we chose the crude MeOH extract of this soft coral for detailed chemical studies, which resulted in the isolation of four new diterpenes, elisabethin E (**2**), elisabethin F (**2**), pseudopterosin P (**3**), and pseudopterosin Q (**4**). We report the isolation and structure elucidation of **1**–**4**. Compounds **3** and **4** showed antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Enterococcus faecalis* at 25 µg/ml. Compounds **1** and **2** were inactive in our antibacterial bioassays at the same dose.

2. Results and Discussion. – Our first compound, elisabethin E (**1**), was isolated as colorless oil. The UV spectrum showed a λ_{max} at 269 nm, indicating the presence of a conjugated homoannular diene π system in the molecule [11]. The IR spectrum of **1** displayed intense absorption bands at 3512 (OH), 2933 (CH), and 1602 (C=C) cm^{-1},



1



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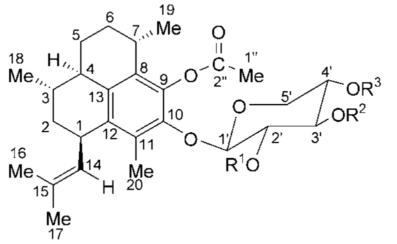
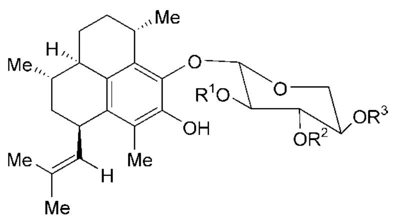
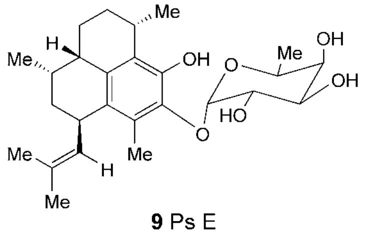


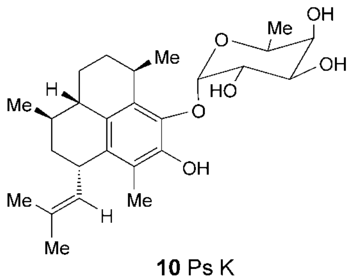
3 Ps P $R^1 = R^2 = R^3 = H$
4 Ps Q $R^2 = Ac$, $R^1 = R^3 = H$



5 Ps A $R^1 = R^2 = R^3 = H$
6 Ps B $R^1 = Ac$, $R^2 = R^3 = H$
7 Ps C $R^2 = Ac$, $R^1 = R^3 = H$
8 Ps D $R^3 = Ac$, $R^1 = R^2 = H$



9 Ps E



10 Ps K

and the high-resolution electron-impact mass spectrum (HR-EI-MS) showed the molecular-ion peak at m/z 300.2093, which provided the molecular formula $C_{20}H_{28}O_2$ (calc. 300.2089). Based on further spectral data, structure **1** was established for this new natural product.

The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of **1** showed two *d* at δ 1.06 ($J = 6.5$ Hz) and 1.19 ($J = 6.8$ Hz) for Me(18) and Me(19), respectively (*Table 1*)[1]. The allylic Me(12), Me(13), and Me(20) protons resonated at δ 1.69, 1.73, and 1.90, respectively. The olefinic signals at δ 5.53 ($J = 4.6$ Hz), 5.10 ($J = 10.0$ Hz), 5.69 ($J = 1.8$ Hz), and 6.00 ($J = 1.8$ Hz) were due to H–C(5), H–C(10), H–C(14), and H–C(16), respectively. H–C(4) resonated at δ 4.35 ($J(4,5) = 5.5$ Hz, $J(4,3) = 4.3$ Hz). Its downfield chemical-shift value indicated the presence of a geminal OH group. The COSY-45° and TOCSY plots showed the presence of two isolated spin systems in **1**.

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[1] Arbitrary numbering; for systematic names, see *Exper. Part.*

Table 1. *NMR Chemical Shift Assignments and* $^1H,^{13}C$ *One-Bond Shift Correlations of All H-Bearing C-Atoms of Compounds* **1** *and* **2**, *as Determined from the HSQC Spectrum*. δ in ppm, *J* in Hz. Arbitrary numbering.

	1		**2**	
	δ (H)	δ (C)[a])	δ(H)	δ(C)[a])
C(1)	–	52.4 (*s*)	–	44.8(*s*)
H–C(2)	2.50 (*m*)	50.3 (*d*)	2.47 (*m*)	50.5 (*d*)
H–C(3)	2.20 (*m*)	42.4 (*d*)	2.18 (*m*)	41.9 (*d*)
H–C(4)	4.35 (*dd*, *J* = 5.5, 4.3)	73.8 (*d*)	3.74 (*ddd*, *J* = 9.8, 5.4, 4.0)	67.3 (*d*)
H–C(5) or $CH_2(5)$	5.53 (*d*, *J* = 4.6)	126.0 (*d*)	2.47 (*m*), 1.88 (*m*)	37.8 (*t*)
C(6) or H–C(6)	–	130.7 (*s*)	3.12 (*m*)	45.2 (*d*)
H–C(7)	2.25 (*m*)	44.8 (*d*)	2.29 (*m*)	43.7 (*d*)
$CH_2(8)$	1.54 (*m*), 1.39 (*m*)	38.3 (*t*)	1.58 (*m*), 1.40 (*m*)	37.9 (*t*)
H–C(9)	2.34 (*m*)	45.6 (*d*)	2.37 (*m*)	44.6 (*d*)
H–C(10)	5.10 (*d*, *J* = 10.0)	127.9 (*d*)	5.08 (*d*, *J* = 9.7)	128.2 (*d*)
C(11)	–	133.8 (*s*)	–	135.6 (*s*)
Me(12)	1.69 (*s*)	17.8 (*q*)	1.68 (*s*)	23.6 (*q*)
Me(13)	1.73 (*s*)	25.4 (*q*)	1.72 (*s*)	25.7 (*q*)
H–C(14)	5.69 (*d*, *J* = 1.8)	128.5 (*d*)	5.92 (*d*, *J* = 1.6)	128.3 (*d*)
C(15)	–	132.4 (*s*)	–	132.5 (*s*)
H–C(16)	6.00 (*d*, *J* = 1.8)	131.3 (*d*)	6.08 (*d*, *J* = 1.6)	131.0 (*d*)
C(17)	–	152.3 (*s*)	–	151.8 (*s*)
Me(18)	1.06 (*d*, *J* = 6.5)	18.4 (*q*)	1.07 (*d*, *J* = 6.4)	18.0 (*q*)
Me(19)	1.19 (*d*, *J* = 6.8)	19.2 (*q*)	1.20 (*d*, *J* = 6.6)	19.1 (*q*)
Me(20)	1.90 (*s*)	9.8 (*q*)	1.92 (*s*)	9.6 (*q*)

[a]) Multiplicity was determined from the DEPT spectrum.

The first spin system **1a** was traced from the olefinic H–C(10) (δ 5.10), which showed vicinal couplings with H–C(9) (δ 2.34) and allylic couplings with Me(12) (δ 1.69) and Me(13) (δ 1.73) (see *Fig. 1,a*). H–C(9) further exhibited COSY-45° interactions with $CH_2(8)$ (δ 1.54 and 1.39), $CH_2(8)$ with H–C(7) (δ 2.25), and H–C(7) with Me(19) (δ 1.19) and H–C(5) (δ 5.53; weak). The TOCSY plot exhibited strong cross-peaks between H–C(7) and H–C(5), representing allylic couplings between them. H–C(5) showed vicinal couplings with H–C(4) (δ 4.35), H–C(4) with H–C(3) (δ 2.20), and H–C(3) with H–C(2) (δ 2.50) and Me(18) (δ 1.06). The second spin system **1b** was traced by the combination of COSY-45° and TOCSY plots, in which H–C(14) (δ 5.69) showed vinylic couplings with H–C(16) (δ 6.00) (see *Fig. 1,a*). H–C(14) and H–C(16) also exhibited allylic couplings with Me(20) (δ 1.90).

The ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) of **1** showed the resonance of all twenty C-atoms, and the DEPT experiment established the multiplicity of each C-signal (*Table 1*), indicating the presence of 5 Me,

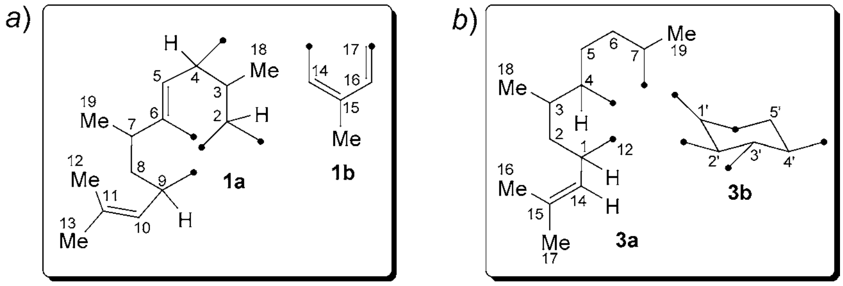
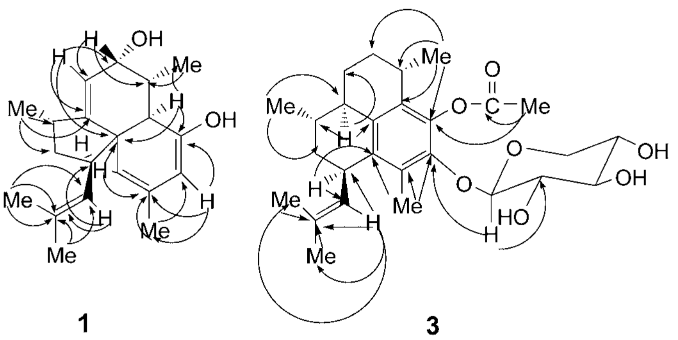


Fig. 1. *Partial structures of* a) *elisabethin E* (**1**) *and* b) *pseudopertosin P* (**3**), *obtained from the COSY-45° and TOCSY plots*

1 CH_2, and 9 CH signals. Subtraction of the DEPT spectrum from the broadband ^{13}C-NMR spectrum of **1** suggested the presence of 5 quaternary C-atoms. Eight olefinic signals at δ 126.0, 130.7, 127.9, 133.8, 128.5, 132.4, 131.3, and 152.3 were due to C(5), C(6), C(10), C(11), C(14), C(15), C(16), and C(17), respectively. The combination of the broadband ^{13}C-NMR and DEPT spectra showed that C(5), C(10), C(14), and C(16) were methine groups, while C(6), C(11), C(15), and C(17) were quaternary C-atoms. This was further confirmed by the HSQC spectrum, in which H–C(5) (δ 5.53), H–C(10) (δ 5.10), H–C(14) (δ 5.69), and H–C(16) (δ 6.00) showed ^{1}H,^{13}C one-bond shift correlations with C(5) (δ 126.0), C(10) (δ 127.9), C(14) (δ 128.5), and C(16) (δ 131.3), respectively. The downfield δ of C(17) (δ 152.3) suggested that this is an OH-bearing C-atom. Another downfield aliphatic signal at δ 73.8 was due to the OH-bearing C(4) and showed cross-peaks with H–C(4) (δ 4.35) in the HSQC spectrum. The presence of an OH group at C(4) was also confirmed by the ^{1}H-NMR spectrum in (D_5)pyridine, in which H–C(4) showed an induced paramagnetic shift from δ 4.35 to δ 4.78. It has been reported that a H-atom at an OH-bearing C-atom experiences an induced paramagnetic shift of *ca.* 0.2 ppm, when the ^{1}H-NMR spectrum is recorded in (D_5)pyridine [12].

The HMBC spectrum allowed to connect the partial structures **1a** and **1b** obtained from the COSY-45° and TOCSY data. H–C(5) (δ 5.53) showed cross-peaks with C(1) (δ 52.4), C(4) (δ 73.8), and C(6) (δ 130.7). H–C(2) (δ 2.50) showed HMBC interactions with C(1) (δ 52.4), C(3) (δ 42.4), C(6) (δ 130.7), and C(17) (δ 152.3). H–C(7) (δ 2.25) exhibited cross-peaks with C(1) (δ 52.4), C(5) (δ 126.0), and C(6) (δ 130.7). H–C(9) (δ 2.34) showed HMBC interactions with C(1) (δ 52.4), C(2) (δ 50.3), and C(6) (δ 130.7). The long-range interactions of H–C(14) (δ 5.69) with C(1) (δ 52.4), C(2) (δ 50.3), and C(6) (δ 130.7) were also observed in the spectrum. These HMBC observations indicated that compound **1** has a tricarbocyclic skeleton. The interpretation of the already discussed NMR data suggested that compound **1** has an elisabethin-like structure. The MS of **1** also favored this structure type, which satisfied the seven degrees of unsaturation as evident from the molecular formula $C_{20}H_{28}O_2$. The tricarbocyclic skeleton with three C=C bonds inside the rings A and C were accounted for six degrees of unsaturation, whereas the seventh degree of unsaturation was satisfied by the presence of a C=C bond in the 2-methylprop-1-enyl side chain at C(9). Important HMBC interactions of **1** are shown in *Fig. 2*.



1 3

Fig. 2. *Important HMBC interactions of compounds* **1** *and* **3**

The configurations of all chiral centers were established with the aid of the NOESY experiment, optical rotation, and ^{1}H-NMR coupling constants. The optical rotation of **1** ($[\alpha]_D^{20} = +39$) was close in magnitude and sign to that of elisabethin D ($[\alpha]_D^{20} = +26.3$) [9], suggesting similar configurations at C(3), C(7), and C(9) of **1** as reported earlier for elisabethins A–D [9]. This was confirmed by the NOESY plot, in which H–C(2) (δ 2.50) showed an NOE with H–C(9) (δ 2.34). H–C(9) exhibited cross-peaks with Me(18) (δ 1.06) and Me(19) (δ 1.19), indicating their *cis* relationship. According to [9] H–C(2), H–C(9), Me(18), and Me(19) have α-orientations in this class of natural products. H–C(4) (δ 4.35) showed NOE cross-peaks with H–C(3) (δ 2.20) and H–C(7) (δ 2.25). These NOESY data helped us to assign the β-orientation for H–C(4) and the α-orientation for OH–C(4). The β-position of H–C(4) was also evident from the ^{1}H-NMR coupling constants ($J(4,5) = 5.5$ Hz, $J(4,3) = 4.3$ Hz). A study of *Dreiding* models of **1** indicated that ring A exists in a twisted chair conformation, in which Me(18) at C(3) is axially oriented and OH–C(4) is equatorially oriented. This was further supported by axial-equatorial coupling constant between H–C(4) and H–C(3) (J = 4.3 Hz), thus suggesting a β-orientation of H–C(4).

Our second compound, elisabethin F (**2**), was also isolated as a colorless oil. Its UV and IR spectra were identical to those of compound **1**. The HR-EI-MS of **2** showed the molecular-ion peak at m/z 302.2243, which provided molecular formula $C_{20}H_{30}O_2$ (calc. 302.2246). Most of the key-fragment patterns in the MS of **2** were the same as those in the MS of **1**, but they appeared at m/z values 2 amu higher indicating that **2** is a dihydro derivative of **1**.

The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) of **2** were nearly identical to those of **1**, except for the resonances arising from the C(5)–C(6) moiety[1]). The ^{1}H-NMR spectrum of **2** showed signals at δ 2.47 and 1.88, which were due to CH_2(5). H–C(6) resonated at δ 3.12. CH_2(5) (δ 2.47 and 1.88) showed vicinal couplings with H–C(4) (δ 3.74) and H–C(6) (δ 3.12) in the COSY-45° plot. The latter showed cross-peaks with H–C(7) (δ 2.29). The remaining observations in the COSY-45° plot of **2** were the same as was those observed for **1**. The ^{13}C-NMR spectrum of **2** showed up-field resonances for C(5) (δ 37.8) and C(6) (δ 45.2), suggesting that C(5) and C(6) were sp^3-hybridized. The MS ^{1}H- and ^{13}C-NMR, HSQC, and HMBC data of **2** were consistent with the structure of a 5,6-dihydro derivative of **1**. The NOESY data of **2** suggested that compound **2** has the same configuration at all chiral centers as established for **1**. H–C(6) (δ 3.12) showed NOEs with H–C(2) (δ 2.47), H–C(9) (δ 2.37), Me(18) (δ 1.07), and Me(19) (δ 1.20), indicating its α-orientation.

Besides **1** and **2**, also the new pseudopterosin P (**3**) was isolated (yellow gum). Its UV spectrum showed a λ_{max} at 280 nm indicating the presence of a highly substituted benzene chromophore [11]. The IR spectrum of **3** displayed intense absorption bands at 3478 (OH), 2904 (CH), 1705 (C=O), 1595 (C=C), and 1100 (C–O) cm^{-1}. The HR-EI-MS of **3** showed a molecular-ion peak at m/z 474.2622, which provided a molecular formula $C_{27}H_{38}O_7$ (calc. 474.2618), indicating the presence of nine degrees of unsaturation in the molecule. Based on further spectral data and comparison with pseudopterins A–L [3–5] and L-xylose, structure **3** was attributed to this new natural product.

The ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of **3** showed 2*d* at δ 1.02 (J = 6.9 Hz) and 1.20 (J = 6.5 Hz) due to Me(18) and Me(19)[1]), respectively, 2*s* at δ 2.01 and 2.09 due to Me(20) and Me(1″), respectively, and 2*s* at δ 1.66 and 1.74 due to the allylic Me(16) and Me(17) protons, respectively (*Table 2*). A downfield *d* for H–C(14) was also observed at δ 5.12 (J = 8.6 Hz). Another 1-H *d* at δ 4.97 (J = 9.0 Hz) was due to the anomeric H–C(1′), the remaining sugar protons resonating at δ 3.75–4.33. Three exchangeable signals at δ 6.27, 7.18, and 8.29 for OH groups disappeared when the ^{1}H-NMR spectrum was recorded in CD_3OD. The COSY-45° plot of **3** revealed the presence of two isolated spin systems. The first spin system **3a** (*Fig. 1,b*) started with the olefinic H–C(14) (δ 5.12), which showed cross-peaks with the allylic Me(16) (δ 1.66), Me(17) (δ 1.74), and H–C(1) (δ 3.58) protons. The latter, in turn, exhibited vicinal couplings with CH_2(2) (δ 1.55 and 1.37), CH_2(2) with H–C(3) (δ 3.26), H–C(3) with Me(18) (δ 1.02) and H–C(4) (δ 3.39), H–C(4) with CH_2(5) (δ 2.01 and 1.51), CH_2(5) with CH_2(6) (δ 1.98 and 1.40), OH_2(6) with H–C(7) (δ 3.58), and H–C(7) with Me(19) (δ 1.20). The second spin system **3b** in the COSY-45° plot was due to a sugar moiety. The anomeric H–C(1′) (δ 4.97) showed cross-peaks with H–C(2′) (δ 3.83), H–C(2′) with H–C(3′) (δ 3.75), H–C(3′) with H–C(4′) (δ 4.01), and H–C(4′) with CH_2(5′) (δ 4.33 and 3.87). The presence of two isolated spin systems in the molecule was also confirmed by the TOCSY experiment (100 ms). The ^{13}C-NMR spectrum ($CDCl_3$, 125 MHz) of **3** showed distinct resonance of all 27 C-atoms. Interpretation of the ^{1}H- and ^{13}C-NMR (*Table 2*), COSY-45°, and HSQC data revealed that **3** has an amphilectane-type diterpene skeleton as aglycone moiety and a xylose sugar attached to it at C(10) as the majority of the signals have chemical-shift values similar to those of pseudopterosins A–L [3–5]. The HSQC spectrum established the ^{1}H,^{13}C one-bond shift correlations in **3** (*Table 2*).

Table 2. *NMR Chemical Shift Assignments and* $^1H,^{13}C$ *One-Bond Shift Correlations of All H-Bearing C-Atoms of Compounds* **3** *and* **4**, *as Determined from the HSQC Spectrum.* δ in ppm, *J* in Hz. Arbitrary numbering.

	3		**4**	
	δ(H)	δ(C)[a])	δ(H)	δ(C)[a])
H−C(1)	3.58 (*m*)	39.4 (*d*)	3.55 (*m*)	39.7 (*d*)
CH_2(2)	1.55 (*m*), 1.37 (*m*)	30.1 (*t*)	1.53 (*m*), 1.36 (*m*)	29.9 (*t*)
H−C(3)	3.26 (*m*)	30.2 (*d*)	3.25 (*m*)	30.0 (*d*)
H−C(4)	3.39 (*m*)	42.7 (*d*)	3.37 (*m*)	43.0 (*d*)
CH_2(5)	2.01 (*m*), 1.51 (*m*)	27.1 (*t*)	1.99 (*m*), 1.53 (*m*)	26.8 (*t*)
CH_2(6)	1.98 (*m*), 1.40 (*m*)	28.1 (*t*)	1.95 (*m*), 1.35 (*m*)	28.5 (*t*)
H−C(7)	3.58 (*m*)	35.6 (*d*)	3.39 (*m*)	35.7 (*d*)
C(8)	–	129.7 (*s*)	–	129.8 (*s*)
C(9)	–	142.0 (*s*)	–	141.8 (*s*)
C(10)	–	145.1 (*s*)	–	145.3 (*s*)
C(11)	–	121.2 (*s*)	–	120.9 (*s*)
C(12)	–	135.0 (*s*)	–	134.7 (*s*)
C(13)	–	129.8 (*s*)	–	130.2 (*s*)
H−C(14)	5.12 (*d*, *J* = 8.6)	129.7 (*d*)	5.14 (*d*, *J* = 8.9)	130.0 (*d*)
C(15)	–	127.6 (*s*)	–	128.2 (*s*)
Me(16)	1.66 (*s*)	21.0 (*q*)	1.65 (*s*)	20.9 (*q*)
Me(17)	1.74 (*s*)	24.0 (*q*)	1.75 (*s*)	24.2 (*q*)
Me(18)	1.02 (*d*, *J* = 6.9)	21.6 (*q*)	1.01 (*d*, *J* = 6.7)	21.4 (*q*)
Me(19)	1.20 (*d*, *J* = 6.5)	25.4 (*q*)	1.19 (*d*, *J* = 6.5)	25.3 (*q*)
Me(20)	2.01 (*s*)	10.8 (*q*)	2.00 (*s*)	11.0 (*q*)
H−C(1′)	4.97 (*d*, *J* = 9.0)	104.5 (*d*)	4.95 (*d*, *J* = 9.4)	104.6 (*d*)
H−C(2′)	3.83 (*dd*, *J* = 9.7, 9.0)	69.6 (*d*)	3.80 (*dd*, *J* = 9.5, 9.4)	70.0 (*d*)
H−C(3′)	3.75 (*dd*, *J* = 10.0, 9.7)	68.6 (*d*)	5.29 (*dd*, *J* = 9.5, 9.3)	74.1 (*d*)
H−C(4′)	4.01 (*ddd*, *J* = 10.0, 9.5, 3.8)	65.9 (*d*)	4.00 (*ddd*, *J* = 9.3, 8.9, 4.0)	67.0 (*d*)
CH_2(5′)	4.33 (br. *t*), 3.87 (br. *t*)	67.7 (*d*)	4.29 (br. *t*), 3.89 (br. *t*)	68.5 (*d*)
Me(1″)	2.09	22.5 (*q*)	2.03 (*s*)	22.8 (*q*)
C(2″)	–	171.3 (*s*)	–	170.8 (*q*)
*Me*CO	–	–	2.07 (*s*)	22.3 (*q*)
Me*C*O	–	–	–	175.8 (*s*)

[a]) Multiplicity was determined from the DEPT spectrum.

The HMBC spectrum of **3** confirmed the assigned connectivities based on the COSY data and the presence of a sugar moiety at C(10) (cross-peak of H−C(1′) (δ 4.97) with C(10) (δ 145.1)). Me(20) (δ 2.01) showed HMBC interactions with C(10) (δ 145.1) and C(11) (δ 121.2). This was further confirmed by the NOESY plot, in which Me(1″) (δ 2.09) showed an NOE with Me(19) (δ 1.20). Me(1″) (δ 2.09) exhibited HMBC interactions with C(2″) (δ 171.3) and C(9) (δ 142.0). Important HMBC interactions of **3** are shown in *Fig. 2*.

The relative configurations at all chiral centers of **3** were established with the aid of NOESY data, which also confirmed the *cis* relationship of Me(18), Me(19), and H−C(4). Me(18) (δ 1.02) showed an NOE with H−C(4) (δ 3.39), which also showed cross-peaks with Me(19) (δ 1.20). The α-orientation of H−C(4), Me(18), and Me(19) was assigned on the basis of chemical-shift comparison of Me(18) and Me(19) of **3** with the reported values in the pseudopterosin series [3–5]. It has been reported that H−C(14) of the aglycone in which the 2-methylprop-1-enyl side chain has an α-orientation resonates at δ 4.97, while a β-oriented 2-methylprop-1-enyl side chain produces a downfield resonance of H−C(14) (δ 5.11) [13]. Since H−C(1) (δ 3.58) of **3** showed an NOE with Me(18) (δ 1.02) and H−C(14) (δ 5.12) and, since the latter resonates close to the H−C(14) of an aglycone with β-oriented 2-methylprop-1-enyl side chain, α-configuration is suggested for H−C(1) and, thus, β-orientation for the 2-methylprop-1-enyl side chain of **3**.

The sugar component of **3** was determined by comparing the ^{1}H- and ^{13}C-NMR data of the sugar moiety with the literature values for pseudopterosins A–L [3–5], which each have a xylose moiety. The L-configuration of the xylose was established by comparing the negative optical rotation of the sugar solution obtained by the hydrolysis of compound **3** by the described protocol [3–5] with the sugar solution of commercially available L-xylose, which was previously treated with a similar amount of an acid. The coupling constant $J(1',2')(=9.0$ Hz) determined the β-L-configuration of this sugar.

Pseudopterosin Q (**4**) was also isolated as a yellow gum. Its UV and IR spectra were identical to those of compound **3**. The HR-EI-MS of **4** showed a molecular-ion peak at *m/z* 516.2420 corresponding to the molecular formula $C_{29}H_{40}O_8$. The combination of ^{1}H- and ^{13}C-NMR, COSY-45°, HSQC, and HMBC data suggested that the new compound **4** is a C(3′) acetyloxy derivative of compound **3**.

The ^{1}H-NMR spectrum of **4** was similar to that of **3**, except for a significant difference in the resonance of H–C(3′) (δ 5.29) of the sugar moiety (*Table 2*), indicating the presence of a geminal acetyloxy group at C(3′). An additional 3-H signal at δ 2.07 was assigned to this MeCO group, which showed ^{13}C-NMR resonances at δ 22.3 (*Me*CO) and 175.8 (Me*C*O). NOESY Data of **4** indicated the same configuration at all chiral centers as established above for **3**. The sugar component in **4** was identified as β-L-xylose, as described for **3**.

Compounds **1**–**4** were tested for antibacterial activity against *Streptococcus pyogenes* (ATCC19615), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25933), and *Pseudomonas aeruginosa* (ATCC 27853) at a dose of 25 μg/ml by using the agar disk diffusion assay [14]. Compounds **3** and **4** exhibited antibacterial activity selectively against the *Gram*-positive bacteria *S. pyogenes*, *S. aureus*, and *E. faecalis*, while both of these compounds were inactive against the *Gram*-negative bacteria *E. coli* and *P. aeruginosa* at the same concentration (*Table 3*). Compounds **1** and **2** exhibited no antibacterial activity against any of the above-mentioned bacteria. Previously, pseudopterosins were reported to exhibit potent anti-inflammatory activity [3–5]. We decided to test also the antibacterial activity of the previously reported pseudopterosins A–L [3–5]. However, we could isolate only the known pseudopterosins A (**5**), B (**6**), C (**7**), D (**8**), E (**9**), and K (**10**) from our extract. Their structures were established by comparing their ^{1}H- and ^{13}C-NMR data with those previously reported [3–5]. Compounds **5**–**10** showed also strong antibacterial activity selectively against *Gram*-positive bacteria (*Table 3*). The *MIC* values of **3**–**10** were measured by using the standard broth dilution antimicrobial susceptibility test [15].

Table 3. *Antibacterial Activity of Compounds* **3**–**10** *against* S. pyogenes, S. aureus, *and* E. faecalis

	S. pyogenes		*S. aureus*		*E. faecalis*	
	Diameter[a]) [mm]	*MIC* [μg/ml]	Diameter[a]) [mm]	*MIC* [μg/ml]	Diameter[a]) [mm]	*MIC* [μg/ml]
3	15	0.8	10	2.0	8	3.5
4	12	1.0	9	2.3	8	3.6
5	15	0.8	11	2.1	9	3.4
6	13	1.0	10	2.3	8	3.2
7	16	1.0	11	2.0	8	3.7
8	17	1.0	9	2.3	9	3.8
9	12	1.0	8	2.3	8	3.6
10	14	1.0	9	2.1	8	3.4

[a]) Diameter of inhibition zone; 25 μg/ml of test compounds were applied to a 6-mm disc.

Conclusions. – We isolated the four new diterpenes **1**–**4** from the soft coral *Pseudopterogorgia elisabethae*. We also discovered that pseudopterosins **3**–**10** exhibited strong antibacterial activity against *Gram*-positive bacteria, and that they were inactive against *Gram*-negative bacteria.

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Experimental Part

General. TLC: silica-gel *GF-254* precoated plates from *Merck*. $[\alpha]_D$: *Polatronic-D* polarimeter (*Hitachi*). UV Spectra: *Shimadzu UV-250 IPC* instrument; λ_{max} in nm. IR Spectra: *Perkin-Elmer 181* spectrophotometer; in cm^{-1}. NMR Spectra: *Bruker AM-500* spectrometer at 500 (^{1}H) and 125 MHz (^{13}C); $CDCl_3$ solns, with $SiMe_4$ as an internal standard; δ in ppm, J in Hz. MS: *Varian MAT-312* double-focussing mass spectrometer connected to a *DEC PDP-11/34* computer system; in m/z (rel. %).

Collection of Marine Organisms. Pseudopterogorgia elisabethae was collected by scuba in 2000 from the Bahamas Islands. The organism was identified by one of us (*G.S.J*), and a voucher specimen has been deposited at *Hauser, Inc.*

Extraction and Isolation of Compounds **1**–**4**. *Pseudopterogorgia elisabethae* was extracted with MeOH. The extract was evaporated to give a gum. The crude extract was redissolved in EtOH/H_2O 2:8 (*v*/*v*). This EtOH/H_2O extract was extracted with AcOEt. After evaporation, the AcOEt extract (100 g) was subjected to column chromatography (silica gel, hexane, 0 → 100% AcOEt/hexane, 0 → 100% MeOH/AcOEt): several fractions. *Fr. A* (obtained with hexane/AcOEt 1:1) was subjected to reversed-phase HPLC (0 → 100% H_2O/MeCN): **1** (3.0 mg) and **2** (4.9) as colorless oils. *Fr. B* (obtained with MeOH/AcOEt 1:99) was subjected to reversed-phase HPLC (0 → 100% H_2O/MeCN): **3** (6.1 mg) and **4** (5.3 mg). Compounds **1**–**4** were homogeneous on TLC in various solvent systems. Compounds **5**–10 were isolated from other fractions obtained from the column chromatography and purified under the previously reported HPLC conditions [4–5].

*(+)-Elisabethin E (=(1*R*,3*S*,5*R*,6*R*,6a*R*,10a*S*)-1,2,3,5,6,6a-Hexahydro-3,6,9-trimethyl-1-(2-methylprop-1-enyl)benz[*d*]indene-5,7-diol;* **1**): 3.1 mg. R_f 0.32. Colorless oil. $[\alpha]^{20}_D = +39$ ($c = 0.58$, $CHCl_3$). UV (MeOH): 232, 269. IR ($CHCl_3$): 3512 (OH), 2933 (CH), 1602 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1.* ^{13}C-NMR ($CDCl_3$, 125 MHz). *Table 1.* HR-EI-MS: 300.2093 (8; $C_{20}H_{28}O_2^{+\cdot}$; calc. 300.2089). EI-MS 300 (10), 284 (7), 269 (25), 254 (40), 55 (100).

*(+)-Elisabethin F (=(1*R*,3*S*,3a*R*,5*S*,6*R*,6a*R*,10a*S*)-1,2,3,3a,4,5,6,6a-Octahydro-3,6,9-trimethyl-1-(2-methylprop-1-enyl)benz[*d*]indene-5,7-diol*; **2**): 3.8 mg. R_f 0.59. Colorless oil. $[\alpha]^{20}_D = +46$ ($c = 0.24$, $CHCl_3$). UV (MeOH): 231, 271. IR ($CHCl_3$): 3478 (OH), 2915 (CH), 1600 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1.* ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 1.* HR-EI-MS: 302.2243 (5; $C_{20}H_{30}O_2^{+\cdot}$; calc. 302.2246). EI-MS: 302 (15), 286 (10), 271 (37), 256 (49), 55 (100).

*(+)-Pseudopterosin P (=(1*S*,3*R*,7*S*,9a*R*)-6-(Acetyloxy)-2,3,7,8,9,9a-hexahydro-1,4,7-trimethyl-3-(2-methylprop-1-enyl)-1*H*-phenalen-5-yl β-L-Xylopyranoside*; **3**): 6.1 mg. R_f 0.28. Yellow gum. $[\alpha]^{20}_D = 122$ ($c = 0.48$, $CHCl_3$). UV (MeOH): 227, 280. IR ($CHCl_3$): 3478 (OH), 2904 (CH), 1705 (C=O), 1595 (C=C), 1100 (C–O). ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1.* ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 1.* HR-EI-MS: 474.2622 (11; $C_{27}H_{38}O_7^{+\cdot}$; calc. 474.2618).

*(+)-Pseudopterosin Q (=(1*S*,3*R*,7*S*,9a*R*)-6-(Acetyloxy)-2,3,7,8,9,9a-hexahydro-1,4,7-trimethyl-3-(2-methylprop-1-enyl)-1*H*-phenalen-5-yl β-L-Xylopyranoside 3-Acetate*; **4**): 5.3 mg. R_f 0.47. Yellow gum. $[\alpha]^{20}_D = 124$ ($c = 0.67$, $CHCl_3$). UV (MeOH): 229, 281. IR ($CHCl_3$): 3470 (OH), 2900 (CH), 1707 (C=O), 1598 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz): *Table 1.* ^{13}C-NMR ($CDCl_3$, 125 MHz): *Table 1.* HR-EI-MS: 516.2420 ($C_{29}H_{40}O_8$, calc. 516.2723).

Hydrolysis of **3** *and* **4**. A soln. of **3** or **4** (3 mg each) in MeOH (5 ml) and 1N HCl (5 ml) was refluxed at 60° for 5 h (TLC monitoring). After the complete consumption of the starting material, the MeOH was evaporated and the aq. layer extracted with AcOEt to remove the aglycone and any unreacted **3** or **4**. The isolated sugar moiety from **3** or **4** had $[\alpha]^{20}_D = -24$ or -28, respectively, *i.e.*, values nearly identical to $[\alpha]^{20}_D = -27$ of commercially available L-xylose treated with a similar amount of an acid.

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