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Isolation and Structure of Providencin: A Highly Oxygenated Diterpene Possessing a Unique Bicyclo[12.2.0]hexadecane Ring System from the Sea Plume *Pseudopterogorgia kallos*

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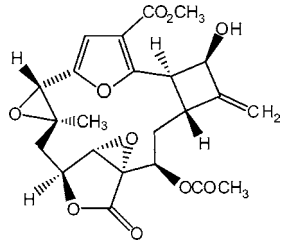
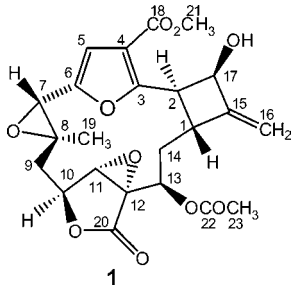
ABSTRACT



Providencin (1) is a naturally occurring cytotoxin isolated from the Caribbean gorgonian octocoral *Pseudopterogorgia kallos*. Its highly oxygenated hexacyclic structure is based on a previously undescribed bicyclo[12.2.0]hexadecane ring system and was established through spectroscopic analysis and X-ray crystallographic analysis. Providencin (1) was shown to exhibit modest anticancer activity against human breast (MCF7), lung (NCI-H460), and CNS (SF-268) cancer cell lines.

The complex molecular architecture and rich functionalization of marine natural products make them utterly attractive targets for total synthesis.[1] In particular, marine diterpenes isolated from the large variety of gorgonian species (sea whips, sea feathers, sea plumes, and sea fans) found within the Caribbean Sea region have for many years captured the attention of synthetic and biosynthetic chemists alike.[2] As part of our continuing chemical investigation of Caribbean gorgonian octocorals, we report here the isolation, structure determination, and biological activity of a novel, highly oxygenated polycyclic diterpene, providencin (**1**).



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The conspicuous sea plume *Pseudopterogorgia kallos* (Bielschowsky, 1918) was collected near Providencia (Old

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48 and previous papers in this series.
(2) (a) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*; VCH: New York, 1996. (c) Kim, A. I.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1267−1270. (d) Heckrodt, T. J.; Mulzer, J. *J. Am. Chem. Soc.* **2003**, *125*, 4680−4681.

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Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data for Providencin (**1**)[a]

position	δ_H, mult (*J* in hertz)	δ_C (mult)	^{1}H−^{1}H COSY	NOESY	HMBC[b]
1	4.68, m	43.1 (CH)	H2, H14α, H16α*β*	H14*β*	H2, H13, H14α*β*, H16α*β*, H17
2	3.47, dd (10.0, 6.1)	42.7 (CH)	H1, H17	H13, H14α, H17	H14α, H17
3		157.9 (C)			H2, H5
4		114.3 (C)			H2, H5
5	6.53, d (1.2)	107.8 (CH)	H7		H7
6		149.0 (C)			H5, H7
7	4.03, br s	55.1 (CH)	H5	H9*β*	H9α, H_3-19
8		56.0 (C)			H7, H9α*β*, H10, H_3-19
9α	2.45, dd (15.3, 2.8)	39.4 (CH_2)	H9*β*, H10	H10, H11, H_3-19	H7, H10, H_3-19
9*β*	2.05, dd (15.3, 4.0)		H9α, H10	H7, H10	
10	4.76, dd (4.0, 2.8)	75.7 (CH)	H9α*β*	H9α*β*, H11	H9*β*, H11
11	3.99, s	60.6 (CH)[c]		H9α, H10, H_3-19	H9*β*, H10, H13
12		58.1 (C)[c]			H10, H11, H14α
13	5.45, br d (10.9)	65.2 (CH)[c]	H14*β*	H2, H14α, H_3-19	H14α
14α	1.73, m	34.7 (CH_2)	H1, H14*β*	H2, H13	H2, H13
14*β*	3.02, m		H13, H14α	H1	
15		151.0 (C)			H14α, H16α*β*, H17
16α	5.21, br d (3.1)	108.6 (CH_2)	H1, H16*β*	H16*β*	
16*β*	5.05, br d (2.2)		H1, H16α	H16α	
17	5.06, br d (6.1)	72.8 (CH)	H2	H2	H2, H16α*β*
18		164.7 (C)			H_3-21
19	1.18, s	20.1 (CH_3)		H9α, H11, H13	H9*β*
20		167.9 (C)			H11
21	3.83, s	51.9 (CH_3)			
22		170.0 (C)			H13, H_3-23
23	2.06, s	20.8 (CH_3)			

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million relative to TMS. ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment. [b] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH} = 6$ and 8 Hz. [c] Broad low-intensity resonance line.

Providence) Island located in the Southwestern Caribbean Sea, among the oceanic islands, atolls, and banks of the San Andrés and Providencia Archipelago, off the Nicaraguan shelf.[3] The sun-dried animal specimens (1.07 kg) were frozen, cut in small pieces, and homogenized exhaustively using a mixture of CH_2Cl_2−MeOH (1:1). After in vacuo concentration, the dried extract (166 g) was subjected to our standard partitioning procedure, resulting in a hexane, $CHCl_3$, and EtOAc fraction.[4] The $CHCl_3$-soluble material (39.3 g) was purified by repeated silica gel flash chromatography and size-exclusion chromatography on a Bio-Beads SX-3 column to yield 20 mg (0.012% dry wt) of pure providencin (**1**). The structure of the new cytotoxin was defined by ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and two-dimensional NOESY experiments. A single-crystal X-ray structure analysis was subsequently carried out in order to confirm the proposed molecular structure of **1**.

Providencin (**1**), $[\alpha]^{20}_D$ +7.9° (*c* 1.2, $CHCl_3$), was isolated as a colorless amorphous solid whose molecular formula of $C_{23}H_{24}O_{10}$ was established on the basis of HREIMS analysis ($[M]^{+\bullet}$; *m/z* 460.1366, +0.0004 mmu),[5] ^{13}C NMR, and DEPT spectra. Thus, 12 degrees of unsaturation were determined for **1**. The IR spectrum of **1** indicated the presence of hydroxyl (ν_{max} 3461 cm^{-1}), ester (ν_{max} 1783, 1743, 1718 cm^{-1}), olefin (ν_{max} 3129, 3078, 1648, 1620, 1577 cm^{-1}), and epoxide (ν_{max} 1235 cm^{-1}) groups, and the UV spectrum (MeOH) showed maxima at λ_{max} 203 (ϵ 11500) and 251 (ϵ 4900) nm. NMR experiments were performed in $CDCl_3$ to enhance peak separation; however, partial peak broadening in the ^{1}H NMR as well as peak splitting in the ^{13}C NMR spectra was observed, suggesting the presence of at least two distinct conformations in solution (Table 1). The ^{13}C NMR spectrum displayed signals for 23 carbons. DEPT analysis indicated that **1** contained three methyls, three methylenes, eight methines, and nine quaternary carbons. The presence of nine sp^2-hybridized carbon atoms in the molecule, as deduced from the ^{13}C and DEPT NMR spectra, corresponding to three carbon−carbon and three carbon−oxygen double bonds as the only multiple bonds, indicated compound **1** to be hexacyclic. An HMQC experiment established all single-bond ^{1}H−^{13}C connectivities.

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), while being generally consistent with the presumption that **1** was a diterpene, showed only three methyl signals, all of which were sharp singlets displaced at δ_H 3.83, 2.06, and 1.18, designated as H_3-21 (a carbomethoxyl), H_3-23 (an acyloxy methyl), and H_3-19. HMQC correlation of δ_H 5.21 (br d, $J = 3.1$ Hz, H_2-16α) and 5.05 (br d, $J = 2.2$ Hz, H_2-16*β*)

(3) For the only in-depth chemical investigation of *P. kallos* prior to this work, see: Look, S. A.; Burch, M. T.; Fenical, W.; Qi-tai, Z.; Clardy, J. *J. Org. Chem.* **1985**, *50*, 5741−5746.

(4) Rodríguez, A. D.; González, E.; Huang, S. D. *J. Org. Chem.* **1998**, *63*, 7083−7091.

(5) EIMS *m/z* 460 $[M]^+$ (29), 442 (100), 400 (75), 382 (36), 367 (49), 218 (69), 167 (98), 136 (90), 90 (31).

with δ_C 108.6 (t, C16) and HMBC correlation (Table 1) of these protons with δ_C 151.0 (s, C15) indicated that **1** contained a strained exocyclic methylene.[6] Eight oxygenated carbon atoms were observed in the ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of **1** in the range 51.9−75.7 ppm, which were assigned to three ester functionalities (an acetate, a γ-lactone, and a carbomethoxyl group), two epoxides, and a hydroxyl linked to a secondary carbon. The acetate was readily identified by the methyl resonance observed in the ^{13}C and ^1H NMR spectra at δ_C 20.8 (q, C23) and δ_H 2.06 (s, 3H, H_3-23), respectively, while the other two ester groups were determined to be a conjugated carbomethoxyl and a nonconjugated γ-lactone, respectively, on the basis of NMR data at δ_C 164.7 (s, C18), 51.9 (q, C21); δ_H 3.83 (s, 3H, H_3-21) and δ_C 167.9 (s, C20), 75.7 (d, C10); δ_H 4.76 (dd, J = 4.0, 2.8 Hz, H-10). The ^{13}C NMR singlets at δ_C 157.9 (C3), 149.0 (C6), and 114.3 (C4) and a doublet at δ_C 107.8 (C5) that was correlated in the HMQC experiment with the ^1H NMR signal at δ_H 6.53 (d, J = 1.2 Hz, H-5), together with the carbomethoxyl signal at δ_H 3.83 (s, 3H, H_3-21) in the ^1H NMR spectrum and at δ_C 164.7 (s, C18) in the ^{13}C NMR spectrum, were assigned to a α,α′-disubstituted β-carbomethoxyfuran constellation.[7] HMQC correlation of δ_H 4.76 (dd, J = 4.0, 2.8 Hz, H-10) with δ_C 75.7 (d, C10) and of δ_H 3.99 (s, H-11) with δ_C 60.6 (d, C11), along with HMBC correlations of H-10 with δ_C 60.6 (d, C11) and 58.1 (s, C12) and of H-11 with δ_C 75.7 (d, C10), 58.1 (s, C12), and 167.9 (s, C20), indicated the presence of a α,β-epoxy γ-lactone moiety.[8] The presence of a trisubstituted epoxide was evident from the ^{13}C NMR singlet at δ_C 56.0 (C8) and a doublet at δ_C 55.1 (C7) that was correlated in the HMQC experiment with the ^1H NMR signal at δ_H 4.03 (br s, H-7). The latter proton was correlated in the HMBC experiment with δ_C 149.0 (s, C6) and 107.8 (d, C5). The methyl at δ_H 1.18 (s, H_3-19) showed HMBC correlations with δ_C 56.0 (s, C8), 55.1 (d, C7), and 39.4 (t, C9), indicating **1** contained a methyl-bearing trisubstituted epoxide flanked by a methylene carbon and a quaternary olefinic carbon. Thus, it quickly became apparent that **1** contained many of the same functionalities present in the bipinnatins, a series of highly oxygenated cembrane-based diterpenes isolated from the gorgonian *Pseudopterogorgia bipinnata*.[9] On the other hand, two conspicuous methine carbon atoms at δ_C 43.1 (d, C1) and 42.7 (d, C2), which were correlated by HMQC with proton signals directly correlated by $^1H-^1H$ COSY at δ_H 4.68 (m, H-1) and 3.47 (dd, J = 10.0, 6.1 Hz, H-2), respectively, were located in a cyclobutane ring along with the exocyclic double bond and secondary hydroxyl group. Additional $^1H-^1H$ COSY correlations observed between H-2 and the signal at δ_H 5.06 (br d, J = 6.1 Hz, H-17), as well as long-range couplings between the terminal methylene protons H_2-16 and H-1, established almost all of the connectivity about the cyclobutane ring. Further analysis of the HMBC experiment (Table 1) provided additional connectivities between all six of the rings within **1** to form a bicyclo[12.2.0]hexadecane system (Figure 1). Some key HMBC correlations establishing this ring system were observed between H-2 to C1, C3, C4, and C17; H-7 to C5, C6, C8, and C9; H-10 to C8, C9, C11, and C12; and H-13 to C1, C11, and C14. Connections from the cyclobutane system to the macrocycle were limited to HMBC correlations from H-2 to C1 and C17 and from H-16α and H-16β to C1, C15, and C17.

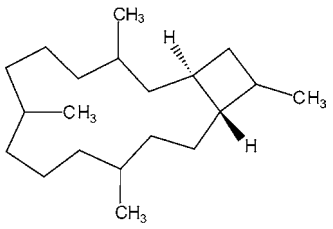

Figure 1. Trans-fused bicyclo[12.2.0]hexadecane ring system demarcates the novel providenciane carbon skeleton in providencin (**1**).

The relative stereochemistry of providencin (**1**) containing nine chiral centers was determined using a combination of NMR methods (COSY, NOESY, and $^1H-^1H$ NMR coupling constants) coupled with a molecular modeling study.[10] The pivotal proton H-2 occurs as a doublet of doublets exhibiting strong couplings with H-1 and H-17. In agreement with the proposed relative stereochemistry, the large axial−axial coupling constant between H-1 and H-2 (J = 10.0 Hz) indicated these protons to be trans coupled, whereas the small axial−equatorial coupling constant between H-2 and H-17 (J = 6.1 Hz) required the latter protons to be cis coupled. The H-1 signal showed a proton COSY correlation only to the H_2-14 signal at δ_H 1.73, which in turn showed no couplings with H-13. If we arbitrarily assume that H-1 is in a pseudoaxial conformation above the plane of the molecule, we can deduce that H-14α (δ_H 1.73) must be below the plane in a trans pseudodiaxial conformation to H-1. As H-13 showed a proton COSY correlation only with the H_2-14 signal at δ_H 3.02 (H-14β), which in turn showed no couplings with H-1, hence H-13 and H-14β must lie in a nearly anti-periplanar conformation (J = 10.9 Hz), placing the acetate group at C13 above the plane in the β configuration. Strong NOESY correlations between H-2, H-14α, and H_3-19 with H-13 established the spatial proximities of these protons on the bottom face of the molecule (Table 1). Similarly, NOESY interactions between H-7 and the H_2-9 signal at δ_H 2.05 (H-9β) allowed the assignment of these protons to the upper face of **1**. On the other hand, pronounced NOESY correlations between H-10, H-11, and H_3-19 with the H_2-9 signal at δ_H 2.45 (H-9α) placed these protons below the plane,

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(7) Rodríguez, A. D.; Soto, J. J. *Chem. Pharm. Bull.* **1996**, *44*, 91−94.
(8) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett.* **1989**, *30*, 3491−3494.
(9) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Nat. Prod.* **1999**, *62*, 1228−1237 and references therein.
(10) Lowest energy conformers were searched using MMFF force field implemented in the MacSpartan Pro program (Wavefunction, Inc.).

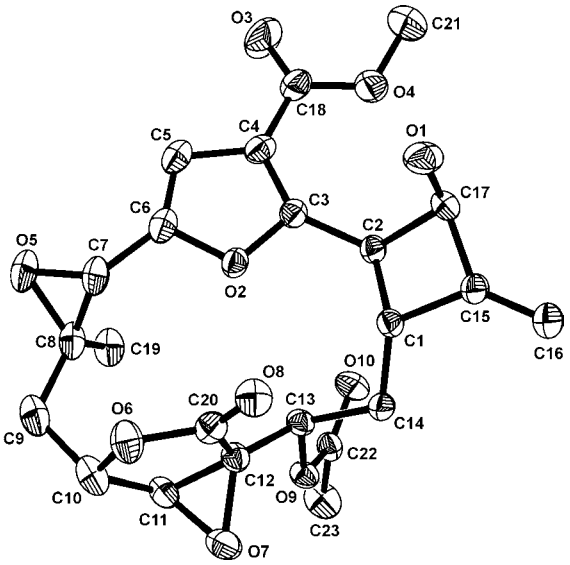

Figure 2. ORTEP diagram with 30% probability of ellipsoids showing the crystallographic atom-numbering scheme and solid state conformation of providencin (**1**). The hydrogen atoms have been omitted for clarity.

resulting in the geometry proposed. The spatial relationships of the γ-lactone, furan, epoxide, and cyclobutane rings within the macrocyclic system was further confirmed by the near absence of coupling ($J < 1.0$ Hz) of the δ_H 3.99 absorption for H-11, which can be attributed to the combined electronegativity effects of vicinal trans coplanar oxygen atoms on the coupling strength of the H-10 and H-11 protons.[11] The dihedral angles between these protons diminish the coupling strength of each proton, reducing their mutual coupling to less than 1 Hz. Thus, the overall relative stereochemistry for providencin (**1**) is 1*R**,2*R**,7*S**,8*R**,10*S**,11*S**,12*S**,13*R**,17*R**.

Recrystallization by slow evaporation in a mixture of 9:1 MeOH/$CHCl_3$ produced good quality crystals of **1**, which allowed a single-crystal X-ray structure analysis to be carried out, thus confirming its molecular structure. The computer-generated perspective drawing of the final X-ray model of **1**, without hydrogens, shown in Figure 2 revealed that the solution conformation is similar to that in the solid state. The absolute configuration of the molecule was not determined in the X-ray experiment. The novel carbon skeleton of providencin (**1**), which we named providenciane, represents a new class of marine-derived diterpenes. A numbering scheme that preserves the C1 to C20 numbering of the parent cembrane skeleton is proposed.

Providencin (**1**) displayed modest in vitro cytotoxicity against MCF7 breast cancer, NCI-H460 nonsmall cell lung cancer, and SF-268 CNS cancer. The percent of growth of the treated cells when compared to the untreated control cells was approximately 57, 39, and 94%, respectively.

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Supporting Information Available: Detailed description of experimental procedures and tables of crystal data for providencin (**1**) (crystal data and structure refinement, atomic coordinates, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates). This material is available free of charge via the Internet at http://pubs.acs.org.

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