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Isolation, Structure Elucidation, and Synthesis of Bisgersolanolide, a Novel Heptacyclic *Bis*-diterpenoid from the Gorgonian Octocoral *Pseudopterogorgia bipinnata*

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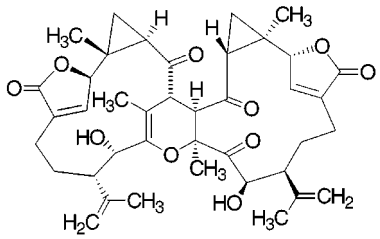
ABSTRACT



A chemical study of the hexane extracts of the Caribbean gorgonian *Pseudopterogorgia bipinnata* (Verrill) collected in San Andrés Island, Colombia, has led to the isolation of an unprecedented heptacyclic C_{40} *bis*-diterpenoid, bisgersolanolide (1). The structure of this novel secondary metabolite, which was established by spectroscopic studies that included 2D NMR correlation methods, IR, UV, and accurate mass measurements, was confirmed by synthesis.

Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* have been a prolific source of chemically and biologically important secondary metabolites.[1,2] The vast majority of identified metabolites from this source are terpenoid compounds, among which the sesquiterpenoids, the diterpenoids, and the steroids predominate.[3] In this Letter we wish to describe the isolation, structure elucidation, and synthesis of an unprecedented heptacyclic *bis*-diterpenoid, bisgersolanolide (**1**).

Bisgersolanolide was isolated from the gorgonian octocoral *Pseudopterogorgia bipinnata* (Verrill) collected near San Andrés Island, Colombia.[4] The dry animal (2.1 kg) was blended with MeOH−$CHCl_3$ (1:1) (5 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (167.5 g). After partitioning the crude extract between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$ (4 × 1 L). The resulting extract was concentrated in vacuo to yield 43.3 g of an oil which was chromatographed over silica gel (400 g) and separated into 30 fractions (I−XXX) on the basis of TLC analyses. Successive purification of fraction X (1.09 g) by size exclusion column chromatography over Bio-Beads SX-3 (eluting with toluene) and normal-phase HPLC [Partisil 10

(1) Fenical, W. *J. Nat. Prod*. **1987**, *50*, 1001−1008.
(2) Faulkner, D. J. *Nat. Prod. Rep*. **1998**, *15*, 113−158 and previous papers in this series.
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(4) For previous chemical studies of *P. bipinnata*, see: (a) Wright, A. E.; Burres, N. S.; Schulte, G. K. *Tetrahedron Lett*. **1989**, *30*, 3491−3494. (b) Culver, P.; Burch, M.; Potenza, C.; Wasserman, L.; Fenical, W.; Taylor, P. *Mol. Pharmacol*. **1985**, *28*, 436−444. (c) Abramson, S. N.; Trischman, J. A.; Tapiolas, D. M.; Harold, E. E.; Fenical, W.; Taylor, P. *J. Med. Chem*. **1991**, *34*, 1798−1804.

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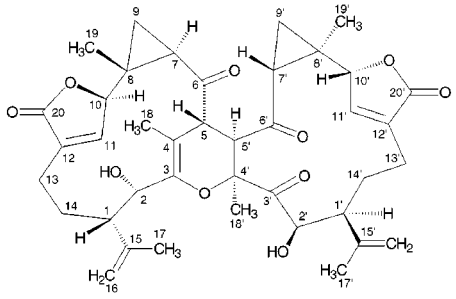
M9/50 eluting with 15% 2-propanol in hexane] afforded 5.6 mg of bisgersolanolide (**1**) (1.3×10^{-2} % of the extract).[5] Compound **1** was obtained as colorless crystals, mp 187−188 °C; $[\alpha]^{24}_D$ +8.4° (*c* 1.30, $CHCl_3$); UV (MeOH) λ_{max} 210 nm (ϵ 51600). A molecular formula of $C_{40}H_{48}O_{10}$ for **1** was determined by HRFABMS [$(M + 1)^+$, *m*/*z* 689.3315, calcd for $C_{40}H_{49}O_{10}$, 689.3325]. The IR spectrum (thin film) indicated the presence of −OH groups (3600−3200, 1088 cm^{-1}), ester (1751, 1281 cm^{-1}) and ketone (1705, 1704, and 1703 cm^{-1}) carbonyls, double bonds (1686, 1651, 1644, 853 cm^{-1}), isopropenyl groups (3081, 1401, 903 cm^{-1}), and a vinyl alkyl ether group (1241 and 1065 cm^{-1}).



Bisgersolanolide (1)

The following fragments were suggested by ^1H and ^{13}C NMR: two ester [δ_C 173.8 (s) and 173.7 (s)] and three ketone [δ_C 207.8 (s), 206.1 (s), and 203.5 (s)] carbonyls, a tetrasubstituted vinyl alkyl ether [δ_C 147.8 (s) and 106.2 (s)], and two disubstituted [δ_C 143.7 (s), 143.5 (s), 114.6 (t), 112.4 (t); δ_H 5.13 (1H, br s), 5.10 (1H, br s), 4.99 (1H, br s), 4.92 (1H, br s)] and two trisubstituted [δ_C 146.4 (d), 146.1 (d), 138.7 (s), 138.6 (s); δ_H 6.78 (2H, br s)] double bonds. Since these functionalities accounted for 10 degrees of unsaturation, bisgersolanolide had to be heptacyclic. Five carbons, not counting the vinyl alkyl ether, were attached to oxygen, most plausibly as two secondary esters [δ_C 84.1 (d), 83.6 (d); δ_H 5.12 (1H, br s), 5.05 (1H, br s)], one tertiary alkyl ether [δ_C 79.4 (s)], and two secondary hydroxyls [δ_C 72.9 (d), 68.8 (d); δ_H 4.91 (1H, br d, J = 8.5 Hz), 4.54 (1H, br s)]. A pair of shielded quaternary carbons were observed at δ 34.2 (s) and 33.9 (s), suggesting the presence of two cyclopropane rings. Six methyl groups attached to quaternary carbons were identified [δ_C 22.8 (q), 22.7 (q), 20.7 (q), 15.0 (q), 14.8 (q), 14.5 (q); δ_H 1.90 (3H, br s), 1.88 (3H, br s), 1.67 (3H, br s), 1.52 (3H, s), 1.42 (3H, s), 1.39 (3H, br s)]. The ^{13}C NMR spectrum also showed six methine and six methylene groups. While the number of methyl groups combined with the parent molecular formula suggested that **1** was terpenoid in origin, the ^1H NMR and ^{13}C NMR spectra unambiguously ruled out a biscembranoid-type structure.[6] No examples of heptacyclic *bis*-diterpenoids of marine origin have been reported in the literature, and since our repeated attempts to obtain suitable crystals for X-ray diffraction were unsuccessful, the structure for **1** was defined by comprehensive 2D NMR experiments involving $^1H-^1H$ COSY experiments and HMQC (1-bond) and HMBC (2- and 3-bond) heterocorrelation measurements. Detailed analyses of these spectra led to assignments of proton connectivities for three distinct ^1H spin systems, subunits **1a**−**c** (Figure 1).

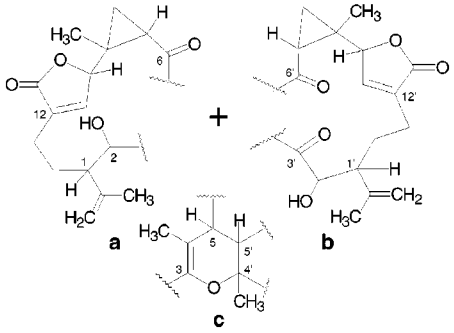


Figure 1. Partial structures of bisgersolanolide (**1**) deduced from HETCOR and $^1H-^1H$ COSY measurements.

The two α,γ-disubstituted butenolide units **1a**,**b** and the 2,3-dihydro-4*H*-pyran unit **1c** were connected together in the proper sequence by HMBC, long-range COSY, and NOESY ^1H NMR data. Thus, fragment **1a** and fragment **1b** were connected to substructure **1c** in the following manner. The site of attachment of carbonyl carbon C6 (resonating at 206.1 ppm) was speculated to be C5, the methine proton of which was shifted downfield to δ 3.36 by virtue of it also being allylic. Direct $^1H-^{13}C$ connectivity linking H5 with C6 was clearly observed from the HMBC spectrum, thus confirming the latter contention. The HMBC spectrum also showed clearly the connection between the C6′ carbonyl and C5′ methine proton. Evidence supporting the linking of C3′ to substructure **1c** through C4′ was provided by additional long-range proton−carbon couplings between the H5′ and Me-18′ protons with the C3′ carbonyl carbon. Since the H1/H2 coupling response in the $^1H-^1H$ COSY spectrum showed no further correlations to link to the other spin systems in the molecule (see Table 1), and no significant HMBC correlations involving H2 were observed, substructure **1c** and the lower end of fragment **1a** were assembled on the basis of NOESY data alone. Thus, a strong NOE response between the oxygen-substituted methine proton H2 and the Me-18 protons established their spatial proximity. These measurements allowed all protons and their respective carbons to be confidently assigned (Table 1). The relative stereochemistry of **1** was determined by further interpretation of the NOESY ^1H NMR experiment and by analysis of the $^1H-^1H$ coupling constants as shown in Table 1. Confirmation of the structures of the three units as well as the sequencing was provided by partial synthesis of **1** (vide infra).

(5) Spectral data for bisgersolanolide (**1**) have been deposited as Supporting Information.

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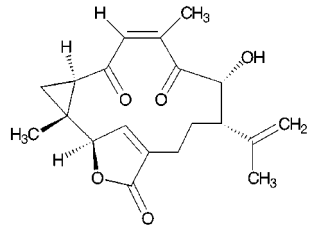
Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Bisgersolanolide (**1**)[a]

position	δ_H, mult, intrgt, (J in Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1	2.16, m, 1H	47.7 (d)	H2, H14αβ	H2, H14β	H16αβ, Me$_{17}$
2	4.54, br s, 1H	68.8 (d)	H1	H1, H14β, Me$_{17}$, Me$_{18}$	
3		147.8 (s)			H5, Me$_{18}$
4		106.2 (s)			H5, Me$_{18}$
5	3.36, d, 1H (12.1)	57.1 (d)	H5′	Me$_{18}$	H5′, Me$_{18}$
6		206.1 (s)			H5, H5′
7	1.96, dd, 1H (6.2, 8.8)	23.4 (d)	H9αβ	H5′, H9α, H11	H10, Me$_{19}$
8		33.9 (s)			H10, Me$_{19}$
9α	1.32, m, 1H	21.5 (t)	H7, H9β		H10, Me$_{19}$
9β	1.32, m, 1H		H7, H9α	H7, Me$_{19}$	
10	5.05, br s, 1H	83.6 (d)	H11	H11, Me$_{19}$ (weak)	H11, Me$_{19}$
11	6.78, br s, 1H	146.1 (d)	H10	H7, H9α, H10	H10, H13αβ
12		138.6 (s)			H10, H11, H13αβ
13α	2.36, m, 1H	21.4 (t)	H14αβ		H11
13β	2.36, m, 1H		H14αβ		
14α	2.21, m, 1H	28.5 (t)	H1, H13αβ	H14β	
14β	1.28, m, 1H		H1, H13αβ	H1, H2, H14α	
15		143.5 (s)			H1, Me$_{17}$
16α	5.13, br s, 1H	112.4 (t)	H16β, Me$_{17}$	H16β, Me$_{17}$	Me$_{17}$
16β	4.92, br s, 1H		H16α, Me$_{17}$	H16α	Me$_{17}$
Me$_{17}$	1.90, br s, 3H	22.8 (q)	H16αβ	H2, H16α	H16αβ
Me$_{18}$	1.39, br s, 3H	14.5 (q)		H2, H5, Me$_{19}$	
Me$_{19}$	1.52, s, 3H	15.0 (q)		H9β, H10 (weak), Me$_{18}$	H9αβ
20		173.7 (s)			H10, H11, H13αβ
1′	2.11, m, 1H	45.6 (d)	H2′, H14′αβ	H2′, H14′α, H16′α	H16′αβ, Me$_{17'}$
2′	4.91, br d, 1H (8.5)	72.9 (d)	H1′	H1′, H14′α, Me$_{17'}$, Me$_{18'}$	
3′		207.8 (s)			H2′, H5′, Me$_{18'}$
4′		79.4 (s)			H5′, Me$_{18'}$
5′	2.97, d, 1H (12.1)	61.4 (d)	H5	H7, Me$_{18'}$	Me$_{18'}$
6′		203.5 (s)			H5′, H9′α
7′	2.01, dd, 1H (5.7, 8.5)	26.9 (d)	H9′αβ	H9′β, H11′	H10′, Me$_{19'}$
8′		34.2 (s)			H10′, Me$_{19'}$
9′α	1.45, br t, 1H (5.3)	20.8 (t)	H7′, H9′β	H9′β, Me$_{19'}$	H10′, Me$_{19'}$
9′β	1.19, dd, 1H (5.0, 8.6)		H7′, H9′α	H7′, H9′α	
10′	5.12, br s, 1H	84.1 (d)	H11′	H11′, Me$_{19'}$ (weak)	H11′, Me$_{19'}$
11′	6.78, br s, 1H	146.4 (d)	H10′	H7′, H9′β, H10′	H10′, H13′αβ
12′		138.7 (s)			H10′, H11′, H13′αβ
13′α	2.31, m, 1H	20.6 (t)	H14′αβ		H11′
13′β	2.31, m, 1H		H14′αβ		
14′α	1.57, m, 1H	25.6 (t)	H1′, H13′αβ	H1′, H2′, H14′β	
14′β	1.85, m, 1H		H1′, H13′αβ	H14′α	
15′		143.7 (s)			H1′, Me$_{17'}$
16′α	5.10, br s, 1H	114.6 (t)	H16′β, Me$_{17'}$	H1′, H16′β	Me$_{17'}$
16′β	4.99, br s, 1H		H16′α, Me$_{17'}$	H16′α, Me$_{17'}$	
Me$_{17'}$	1.88, br s, 3H	20.7 (q)	H16′αβ	H2′, H16′β	H16′αβ
Me$_{18'}$	1.67, br s, 3H	22.7 (q)		H2′, H5′	
Me$_{19'}$	1.42, s, 3H	14.8 (q)		H9′α, H10′ (weak)	H9′αβ
20′		173.8 (s)			H10′, H11′, H13′αβ

[a] Spectra were recorded in $CDCl_3$ at room temperature. Chemical shift values are in ppm relative to TMS. [b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 and 8 Hz.

Bisgersolanolide (**1**) is the first *bis*-diterpenoid of this structural type, and although its biosynthesis is not known, the fact that diterpenes of the gersolane class have been found in *P. bipinnata* suggests that a plausible biogenesis would involve generation of the 2,3-dihydro-4*H*-pyran ring by a Diels−Alder coupling of two gersolanes. This would generate the novel bisgersolane carbon skeleton of **1** in an efficient manner.[7] The most obvious precursors suggested by this scheme are two molecules of pinnatin C (**2**), a natural product previously identified in this gorgonian species.[8] The concomitance of **1** and **2** strongly implies that **2** is a biogenetical precursor of bisgersolanolide. Therefore, it was interesting to investigate the biogenetic Diels−Alder reaction leading to the synthesis of **1** in vitro. The Diels−Alder dimerization reaction of pinnatin C (**2**) was carried out in toluene-d_8, and the reaction was monitored by ^{1}H NMR spectroscopy. After

confirmation of the appearance of new signals ascribable to a Diels−Alder adduct, at 25 °C for 4 weeks, the mixture was heated at 40−80 °C for 4 h to facilitate the reaction. It should be mentioned that the natural product **1** is one of eight possible Diels−Alder adducts from substrate **2**.[9] If the proposed Diels−Alder reaction had an endo transition state, the stereochemistries at C4′, C5′, and C5 would be those observed in **1**.[10] Besides a minor product, whose presence was detected on TLC but whose structure has not yet been determined, only one compound could be isolated, which was indeed compound **1**. The ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) of the product **1** were consistent with the data reported here. The specific optical-rotation values of the samples from both origins, synthetic and natural, of compound **1** were the same within experimental error. Since our starting material is optically pure without ambiguity in the absolute configuration, this derivation to compound **1** has clarified its absolute configuration as that depicted. Bisgersolanolide is the first *bis*-diterpenoid derived from a classical Diels−Alder dimerization reaction involving a diterpenoid enedione precursor. The fact that the Diels−Alder reaction of pinnatin C (**2**) proceeded stereoselectively under nearly physiological temperatures suggests that the natural product bisgersolanolide (**1**) could be formed nonenzymatically within the bodies of the gorgonian.



Pinnatin C (2)

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and ^{1}H−^{1}H COSY, NOESY, HMQC, HMBC, and HRFABMS spectral data for bisgersolanolide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Among natural products, there are many compounds which are regarded as being formed via cycloaddition processes in vivo. The condensation of two diterpenes forming a C_{40} *bis*-diterpenoid is rare, but several examples have been reported. Compounds of this type can be dimers formed by two units of the same C_{20} skeleton or *bis*-diterpenoids produced by the coupling of two different C_{20} skeletons. On structural grounds, the majority of these *bis*-diterpenes appear to be derived from a classical Diels−Alder reaction of a diterpenoid diene with an activated dienophile-containing diterpenoid precursor. For well-documented examples, see: (a) Falshaw, C. P.; King, T. J. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1749−1752. (b) Pelletier, S. W.; Kapadi, A. H.; Wright, L. H.; Page, S. W.; Newton, M. G. *J. Am. Chem. Soc.* **1972**, *94*, 1754−1755. (c) Hasan, C. M.; Healey, T. M.; Waterman, P. G. *Phytochemistry* **1985**, *24*, 192−194. (d) Pinto, A. C.; Pizzolatti, M. G.; Epifanio, R. de A.; Frankmölle, W.; Fenical, W. *Tetrahedron* **1997**, *53*, 2005−2012.

(8) Rodríguez, A. D.; Shi, J.-G.; Huang, S. D. *J. Org. Chem.* **1998**, *63*, 4425−4432.

(9) If either the diene or the dienophile is chiral and optically active, facially selective addition will give rise to a nonstatistical mixture of optically active diastereomers.

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