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A Marine Diterpene with a Novel Tetracyclic Framework from the West Indian Gorgonian Octocoral *Pseudopterogorgia elisabethae*

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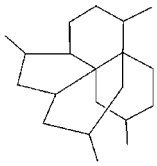
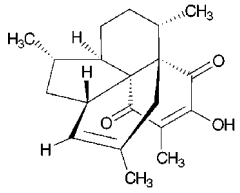
ABSTRACT



the colombiane carbon skeleton

Colombiasin A (1) was isolated from an extract of the West Indian gorgonian octocoral *Pseudopterogorgia elisabethae* that showed strong inhibitorial activity against *Mycobacterium tuberculosis* H37Rv. Structure elucidation by interpretation of 2D-NMR spectroscopic data, IR, UV, and accurate mass measurements (HREI-MS) revealed that colombiasin A belongs to a previously undescribed class of C_{20} rearranged diterpenes possessing an intricate tetracyclic framework.

West Indian corals of the order Gorgonaceae have proved to be a rich source of secondary metabolites with unusual structural features as well as interesting biological activities.[1] Recently, we have initiated a program to discover marine natural products that effect potent inhibitory activity against *Mycobacterium tuberculosis* H37Rv, the aetiological agent that causes tuberculosis. In the screening of a number of extracts, we encountered a gorgonian octocoral, *Pseudopterogorgia elisabethae* (Bayer, 1961), collected off San Andrés Island, Colombia, whose hexane extract exhibited significant activity against *M. tuberculosis*.[2] Bioassay-guided isolation afforded two active benzoxazole alkaloids whose isolation, structure elucidation, and in vitro antituberculosis activity we recently reported.[3] Motivated by the strong inhibitorial activity exhibited by these metabolites, we undertook the acquisition of additional quantities for further biological evaluation. In the course of purifying larger quantities of the hexane extract for additional bioassays, we isolated and identified smaller quantities of a new metabolite possessing an unprecedented carbon skeleton, colombiasin A (**1**), the subject of this report.[4]



colombiasin A (**1**)

The MeOH−$CHCl_3$ (1:1) extract of the gorgonian (∼1 kg of dry wt) was partitioned between hexane and water (3 × 800 mL) to yield a green residue (284 g). A portion of the hexane extract (50 g) was dissolved in a small volume of toluene, filtered, and loaded onto a large Bio-Beads SX-3

(1) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618 and references therein.

(2) For previous research on the chemistry of *P. elisabethae*, see: (a) Schmitz, F. J.; Campbell, D. C.; Kubo, I. *Steroids* **1976**, *28*, 211−221. (b) Look, S. A.; Fenical, W.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5140−5145. (c) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001−1008. (d) Harvis, C. A.; Burch, M. T.; Fenical, W. *Tetrahedron Lett.* **1988**, *29*, 4361−4364. (e) Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 4916−4922.

(3) Rodríguez, A. D.; Ramírez, C.; Rodríguez, I. I.; González, E. *Org. Lett.* **1999**, *1*, 527−530.

(4) Taken in part from the Ph.D. Dissertation of C. Ramírez, University of Puerto Rico, in preparation.

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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 of Colombiasin A (**1**)[a]

position	δ_H, mult (J in Hz)	δ_C (mult)	^{1}H−^{1}H COSY	NOESY	HMBC[b]
1		64.0 (s)			H6, H8β, H10, H12β
2		51.6 (s)			H12β, Me-18
3	1.93, m	33.6 (d)	H4$\alpha\beta$, Me-18	H5β, H9, Me-18	H12β, Me-18
4α	1.30, m	31.1 (t)	H3, H4β, H5$\alpha\beta$	H4β	Me-18
4β	1.59, m		H3, H4α, H5$\alpha\beta$	H4α	
5α	1.83, m	31.8 (t)	H4$\alpha\beta$, H5β, H6	H5β	H4α, H6
5β	1.25, m		H4$\alpha\beta$, H5α, H6	H3, H5α, H7, H9	
6	1.82, m	39.5 (d)	H5$\alpha\beta$, H7	Me-19	H5β, H7, Me-19
7	1.86, m	48.2 (d)	H6, H8$\alpha\beta$, Me-19	H5β, Me-19	H6, H8$\alpha\beta$, Me-19
8α	1.93, m	33.5 (t)	H7, H8β, H9	H8β, Me-19	H7, Me-19
8β	2.13, ddd (2.5, 9.0, 11.7)		H7, H8α, H9, Me-19	H8α, H9	
9	3.05, br m	38.7 (d)	H8$\alpha\beta$, H12$\alpha\beta$, Me-13	H3, H5β, H8β	H7, H8α, H10
10	5.68, br s	123.9 (d)	H12$\alpha\beta$, Me-13	Me-13	H12$\alpha\beta$, Me-13
11		128.9 (s)			H12$\alpha\beta$, Me-13
12α	1.91, br d (18.5)	36.3 (t)	H9, H10, H12β, Me-13	H12β	H10, Me-13
12b	2.41, br d (18.5)		H9, H10, H12α, Me-13	H12α, Me-13, Me-18	
13	1.57, br s	22.8 (q)	H9, H10, H12$\alpha\beta$	H10, H12β	H10, H12α
14		202.6 (s)			H6, Me-20
15		120.4 (s)			Me-20, 16-OH
16		149.5 (s)			Me-20, 16-OH
17		199.6 (s)			H3, 16-OH
18	1.37, d (7.0)	17.7 (q)	H3	H3, H12β	H4β
19	0.81, d (7.3)	22.1 (q)	H7, H8β	H6, H7, H8α	H7, H8α
20	1.90, s	9.7 (q)			
16-OH	6.91, br s[c]				

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million (ppm) relative to TMS. ^{13}C NMR multiplicities were obtained by Attached Proton Test (APT) and DEPT experiments. [b] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz. [c] Exchangeable proton.

column with toluene as eluant. Four fractions were obtained: fraction 1 (24.1 g), fraction 2 (9.2 g), fraction 3 (15.1 g), and fraction 4 (1.57 g). After preliminary NMR analyses, fraction 3 was separated further into 18 fractions by silica gel (270 g) column chromatography using 10% EtOAc in hexane as eluant. Fractions 3.11 and 3.12 were combined (total wt = 4.30 g) and purified by column chromatography on silica gel (150 g) using a step gradient of 10−20% acetone in hexane as eluant. A total of 12 subfractions (A−L) were obtained. Colombiasin A (**1**) (12.6 mg; 1.58×10^{-2} % yield) was obtained pure after subfraction D (167.1 mg) was chromatographed successively over silica gel (7.0 and 1.0 g, respectively) using as eluant 5% EtOAc in hexane and 30% $CHCl_3$ in hexane.[5]

Colombiasin A (**1**) is a yellowish oil that analyzed for $C_{20}H_{26}O_3$ on the basis of its combined HREI-MS ([M$^+$] m/z 314.1879, Δ 0.3 mmu) and ^{13}C NMR spectral features (Table 1). The IR spectrum of **1** showed broad absorptions for a hydroxyl (3376 cm^{-1}) functionality and multiple conjugated carbonyl (1661 cm^{-1}) groups. The UV spectrum showed absorption maxima at 209 and 288 nm, typical for an enedione functionality which was substantiated by the IR absorption at 1661 cm^{-1}. In fact, ^{1}H NMR signals at δ 6.91 (1H, br s, 16-OH) and 1.90 (3H, br s, Me-20), together with ^{13}C signals at δ 202.6 (s, C-14), 199.6 (s, C-17), 149.5 (s, C-16), 120.4 (s, C-15), 64.0 (s, C-1), 51.6 (s, C-2), and 9.7 (q, C-20) are reminiscent of the same substituted cyclohexene-1,4-dione ring system usually found in metabolites of the elisabethane class of diterpenes.[6]

The ^{1}H NMR spectrum showed two singlet methyl signals at δ 1.90 (Me-20) and 1.57 (Me-13), and two doublet methyls at δ 1.37 (J = 7.0 Hz, Me-18) and 0.81 (J = 7.3 Hz, Me-19). There were five methines, two of which were deshielded [δ 5.68 (br s, H-10) and 3.05 (br m, H-9)] and three were shielded [δ 1.93 (m, H-3), 1.86 (m, H-7) and 1.82 (m, H-6)], four pairs of diastereotopic methylenes [δ 1.30/1.59 (m, H-4$\alpha\beta$), 1.25/1.83 (m, H-5$\alpha\beta$), 1.93/2.13 (m, H-8$\alpha\beta$), and 1.91/2.41 (br d, H-12$\alpha\beta$)], and one exchangeable proton [δ 6.91 (16-OH)], in addition to two nonconjugated olefin carbons [δ 128.9 (s, C-11) and 123.9 (d, C-10)]. These NMR data along with other spectral data accounted for four (i.e., the enedione and olefin functionalities) out of eight degrees of unsaturation, thereby suggesting the tetracyclic nature of **1**. Detailed analysis of ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY NMR data confirmed the structure of colombiasin A (**1**) and permitted assignment of all proton and carbon resonances (Table 1).

(5) Colombiasin A (**1**): $[\alpha]^{25}_D$ −55.3° (c 0.9, $CHCl_3$); UV (MeOH) λ_{max} 209 nm (ϵ 6600), 288 nm (ϵ 4000); IR (film) 3376, 2959, 2928, 2871, 2855, 1661, 1454, 1379, 1349, 1152, 1108, 1096, 1042, 1024, 968 cm^{-1}; HREI-MS m/z [M$^+$] calcd for $C_{20}H_{26}O_3$ 314.1882, found 314.1879 (84), 299.1679 (34, $C_{19}H_{23}O_3$), 286.1953 (40, $C_{19}H_{26}O_2$), 271.1674 (27, $C_{18}H_{23}O_2$), 259.1312 (17, $C_{16}H_{19}O_3$), 253.1574 (20, $C_{18}H_{21}O$), 243.1025 (27, $C_{15}H_{15}O_3$), 206.0939 (100, $C_{12}H_{14}O_3$), 201.1646 (28, $C_{15}H_{21}$), 145.1018 (66, $C_{11}H_{13}$), 128.0619 (38, $C_{10}H_8$), 115.0543 (40, C_9H_7), 105.0704 (57, C_8H_9), 93.0688 (41, C_7H_9), 91.0544 (82, C_7H_7), 83.0127 (71, $C_4H_3O_2$), 77.0391 (59, C_6H_5), 55.0544 (49, C_4H_7).

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

Interpretation of the $^1H-^1H$ COSY spectrum of **1**, together with the NOESY spectrum, readily allowed only one independent spin system to be identified in colombiasin A. This spin system consisted of the C-3 methyl group, which correlated to the C-3 proton, which itself correlated along the chain through C-4, C-5, C-6, C-7, C-8, and last to the methine proton at C-9.[7] The C-7 methyl group showed correlations to the C-7 proton, and the latter correlated to the methine proton at C-6 and the diastereotopic protons at C-8. The extensive NMR experiments including long-range $^1H-^1H$ COSY, HMQC, and HMBC led to three partial structures **a**−**c** (Figure 1). Long-range homonuclear couplings, especially via four bonds, provided additional connectivities within unit **a**. Thus, H-10 can be correlated by cross-peaks with Me-13 and the two protons at C-12; the corresponding signals are at δ 2.41 and 1.91 and have a common cross-peak with Me-13 as well. The latter partial connectivity was confirmed by HMBC data (H-12$\alpha\beta$/C-11, Me-13/C-11, H-10/C-12, and H-10/C-13). Similarly, the upper and lower ends of unit **a** were connected through C-2 on the basis of HMBC cross-peaks between H-12β/C-2, H-12β/C-3, and Me-18/C-2.

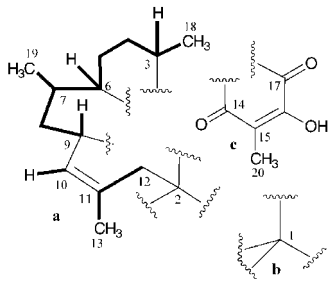


Figure 1. Partial structures of colombiasin A (**1**). The bold lines indicate discrete spin systems identified by $^1H-^1H$ COSY and NOESY NMR experiments.

Connectivities of partial structures **a** and **b** were inferred from key HMBC cross-peaks between protons H-6, H-8β, H-10, and H-12β with C-1, thereby indicating that C-2, C-6, and C-9 must all be connected through C-1. This allowed the construction of rings A, B, and C (Figure 2). To satisfy the molecular formula, the remaining unit **c** must be linked to ring A (or C) via C-1 and C-2, the only connecting points remaining; but there are two conceivable combinations. Fortunately, HMBC correlations between H-6/C-14 and H-3/C-17 allowed us to readily complete ring D which also served to establish the gross structure of **1**. Thus, colombiasin A is a rigid polycyclic diterpene having two adjacent quaternary carbons (C-1 and C-2) one of which (C-1) is intricately connected to all four carbocycle systems. Colombiasin A (**1**) is without precedent in the natural products literature, and its novel skeleton, named colombiane, represents a new class of diterpenes (Figure 2).

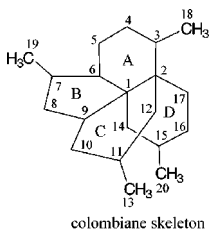
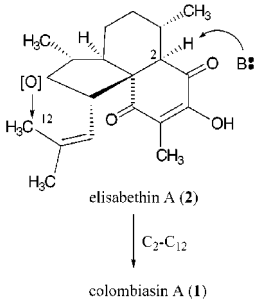


Figure 2. Novel terpenoid carbon skeleton of colombiasin A (**1**) with proposed name, rings, and numbering system.

The relative stereochemistry of colombiasin A (**1**) (1*S**,2*S**,3*S**,6*R**,7*S**,9*S**) was confidently assigned on the basis of NOESY correlation experiments (Table 1). NOESY correlations between H-3/H-9 and H-5β/H-9 quickly established that the A and B rings adopt, respectively, the chair and envelope configuration. Similarly, NOE correlations between H-6/Me-19, H-8α/Me-19, and H-12β/Me-18 indicated the α orientation of both Me-18 and Me-19 as well as the *cis* junction for rings A/B, A/C, A/D, and C/D, and the *trans* junction for rings B/C. In ring C (which adopts a half-chair conformation), correlations between H-10 and Me-13 established their respective proximities and defined their *cis* relationship. Conspicuously absent from the NOESY spectra of **1** were correlations for the signals ascribed to Me-20 and 16-OH which indicated that the D ring is oriented outward beneath the molecule. These overall observations also proved to be consistent with the apparent lack of coupling between H-9/H-10, which according to estimates from a molecular modeling study, require a dihedral angle approaching 90°. Experimental data were not obtained to define the absolute stereochemistry of colombiasin A.

Although the biosynthesis of colombiasin A (**1**) is not known, the fact that diterpenes of the elisabethane class have been found in *P. elisabethae* suggests a common biosynthetic pathway.[8] For instance, the concomitance of elisabethin A (**2**), which was defined by X-ray methods,[6] and colombiasin A (**1**) strongly implies that **2** is a biogenetical precursor of **1**.[9] In such case, elisabethin A (**2**) must first undergo hydroxylation at C-12 followed by phosphorylation or protonation of the new oxygen, based-catalyzed removal of the proton on C-2, and intramolecular alkylation of the resulting enolate (Scheme 1). Further biological studies on colombiasin A are in progress.

Scheme 1



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(7) Since the $^1H-^1H$ COSY spectrum of **1** revealed no coupling between H-9 and H-10, we firmly established their connectivity on the basis of a strong HMBC correlation between H-10 (δ 5.68) and C-9 (δ 38.7).

(8) (a) Rodríguez, A. D.; Ramírez, C.; Rodríguez, I. I. *Tetrahedron Lett*. **1999**, *40*, 7627−7631. (b) Rodríguez, A. D.; Ramírez, C.; Rodríguez, I. I. *J. Nat. Prod*. **1999**, *62*, 997−999. (c) Rodríguez, A. D.; Ramírez, C.; Rodríguez, I. I.; Barnes, C. L. *J. Org. Chem*. **2000**, *65*, in press.

(9) The absolute stereochemistry of elisabethin A (**2**) is not known.