The Cumbiasins, Structurally Novel Diterpenes Possessing Intricate Carbocyclic Skeletons from the West Indian Sea Whip *Pseudopterogorgia elisabethae* (Bayer)[1]

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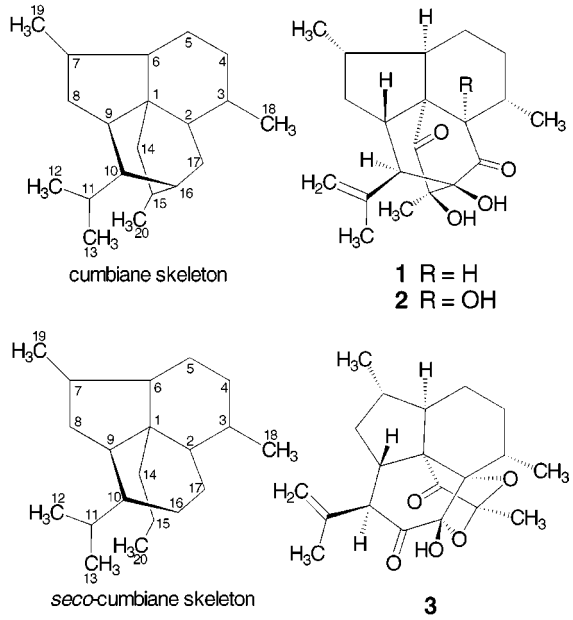
From the hexane extract of the West Indian gorgonian *Pseudopterogorgia elisabethae*, two diterpenes, cumbiasins A (**1**) and B (**2**), having a novel tetracyclic carbon skeleton named cumbiane, have been isolated. In addition, we have isolated cumbiasin C (**3**), a ring cleavage product of cumbiasin B that possesses an unusual carbocyclic framework named *seco*-cumbiane. The structures and relative configurations of metabolites **1**−**3** were elucidated by interpretation of overall spectral data, which included 2D NMR correlation methods, IR, UV, and accurate mass measurements (HREI-MS and HRFAB-MS). The carbocyclic skeletons of the cumbiasins are unprecedented and represent new classes of C_{20} rearranged diterpenes. Cumbiasins A and B display mild in vitro antituberculosis activity.

Introduction

Gorgonian octocorals (sea whips, sea feathers, and sea fans; phylum Cnidaria, order Gorgonacea) of the genus *Pseudopterogorgia* have been recognized as a source of novel secondary metabolites with unique structures.[3] In our continuing search for pharmacologically active metabolites from marine invertebrates collected within the Caribbean region of the West Indies, we have investigated the gorgonian *Pseudopterogorgia elisabethae* (Bayer) and isolated a series of bioactive terpenes with novel carbon skeletons.[4] Here we describe the isolation and structure elucidation of three novel polycyclic diterpenes, cumbiasins A−C (**1**−**3**), which were isolated from the hexane extract of a specimen of *P. elisabethae* collected in 1996 off San Andrés Island, Colombia. Compounds **1** and **2**, containing the novel carbon framework "cumbiane", showed mild growth inhibitory activity in the U.S. Tuberculosis Facility (TAACF) assay employing *Mycobacterium tuberculosis* $H_{37}Rv$.



Results and Discussion

After filtration, the 1:1 $CHCl_3$ and MeOH extract of dry *P. elisabethae* (1.0 kg) was subjected to gel filtration chromatography (Bio-Beads SX-3, toluene) followed by repetitive normal-phase and reversed-phase SiO_2 chromatography to afford three polycyclic diketones named cumbiasin A (**1**) (7.6 mg; 9.53×10^{-3}% dry wt), cumbiasin B (**2**) (6.7 mg; 8.40×10^{-3}% dry wt), and cumbiasin C (**3**) (9.0 mg; 1.13×10^{-2}% dry wt). The structures of these metabolites were determined by interpretation of the 1D and 2D NMR (^{13}C, ^{1}H, $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY), IR, UV, and accurate mass measurements (HREI-MS and HRFAB-MS).

Cumbiasin A (**1**) had the molecular formula $C_{20}H_{28}O_4$ as revealed by HREI-MS *m*/*z* 332.1998 [M^+] (calcd for

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(3) (a) Fenical, W. *J. Nat. Prod.* **1987**, *50*, 1001−1008. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55 and previous reports in this series. (c) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571−4618 and references therein.

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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 Cumbiasin A (1) in $CDCl_3$[a]

position	δ_H, mult, intgr, (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}HCOSY	NOESY	HMBC[c]
1		57.9 (s)			H2, H3, H6, H7, H8β, H9
2	2.23, d, 1H (10.7)	52.9 (d)		H4α, Me-18	H9, Me-18
3	2.18, m, 1H	30.7 (d)	H4$\alpha\beta$, Me-18	H5β, H9, Me-18	H2, Me-18
4α	0.98, m, 1H	34.1 (t)	H3, H4β, H5$\alpha\beta$	H2	H5α, Me-18
4β	1.60, m, 1H		H3, H4α, H5$\alpha\beta$		
5α	1.86, br m, 1H	30.6 (t)	H4$\alpha\beta$, H5β, H6		H6, H7
5β	1.13, m, 1H		H4$\alpha\beta$, H5α, H6	H3, H7, H9	
6	2.21, m, 1H	40.8 (d)	H5$\alpha\beta$, H7	Me-19	H5β, H7, H8$\alpha\beta$, H9, Me-19
7	1.72, m, 1H	40.4 (d)	H6, H8$\alpha\beta$, Me-19	H5β, H8β, H9, Me-19	H5β, H6, H8$\alpha\beta$, H9, Me-19
8α	0.61, ddd, 1H (9.2, 12.5, 12.5)	40.9 (t)	H7, H8β, H9	H10, Me-19	H6, H7, H10, Me-19
8β	2.31, m, 1H		H7, H8α, H9	H7	
9	2.34, m, 1H	41.4 (d)	H8$\alpha\beta$, H10	H3, H5β, H7, H12α	H2, H8α, H10
10	2.54, d, 1H (7.5)	50.6 (d)	H9, H12$\alpha\beta$	H8α, H12α, Me-20	H8$\alpha\beta$, H9, H12$\alpha\beta$, Me-13, 16-OH
11		143.8 (s)			H9, H10, Me-13
12α	4.68, br s, 1H	114.5 (t)	H10, H12β, Me-13	H9, H10, H12β	H10, Me-13
12β	4.93, br s, 1H		H10, H12α, Me-13	H12α, Me-13	
13	1.68, br s, 3H	22.5 (q)	H12$\alpha\beta$	H12β	H10, H12$\alpha\beta$
14		210.0 (s)			H2, H6, H9, Me-20
15		82.7 (s)			H-10, 16-OH, Me-20
16		77.2 (s)			H-10, 16-OH, Me-20
17		212.8 (s)			H2, H10, 16-OH
18	1.34, d, 3H (6.3)	22.0 (q)	H3	H2, H3	H2, H3
19	1.07, d, 3H (7.0)	21.0 (q)	H7	H6, H7, H8α	H8$\alpha\beta$
20	1.48, s, 3H	17.9 (q)		H10	
15-OH	2.17, br s, 1H				
16-OH	3.69, br s, 1H				

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

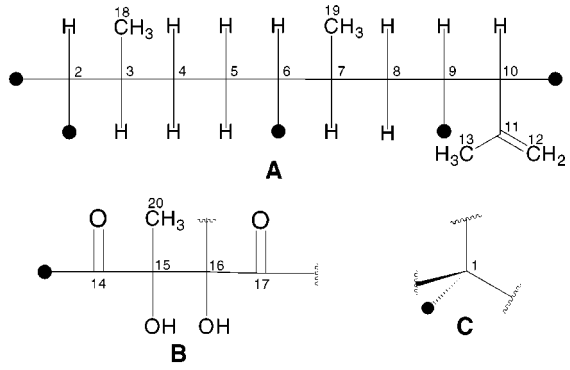


Figure 1. Partial structures **A**−**C** leading to the structure of cumbiasin A (**1**) generated from ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectral data.

$C_{20}H_{28}O_4$ 332.1987) and overall NMR information. This result was subsequently validated on the basis of LRFAB-MS *m*/*z* 333 $[M + H]^+$ and *m*/*z* 355 $[M + Na]^+$. IR absorptions at 3461, 3083, and 1729 cm^{-1} indicated the presence of hydroxyl(s), olefin, and carbonyl group(s), respectively. ^{1}H and ^{13}C NMR data (Table 1) disclosed the presence of two exchangeable protons, two ketone carbonyls, a 1,1-disubstituted olefin, three sp^3 quaternary carbons, two of which were oxygen-bearing, six sp^3 methines, three sp^3 methylenes, and four methyl groups. Since three out of seven unsaturations were accounted for, cumbiasin A (**1**) was inferred to contain four rings. Interpretation of the ^{1}H−^{1}H COSY and TOCSY spectra revealed the proton connectivities of partial structure **A** from H-2 through H_3-13, including vicinal couplings from H-3 to Me-18 and H-7 to Me-19 and long-range correlations from H-10 and H_2-12 to H_3-13 (Figure 1). A combination of heteronuclear 2D NMR techniques along with biogenetic considerations (vide infra) guided the construction of substructure **B** in cumbiasin A (**1**). In this way, using data obtained from HMQC and HMBC experiments, correlations from methyl protons (H_3-13, H_3-18, H_3-19, and H_3-20), in particular, led to the confident assignment of these substructures. Although all 28 hydrogens and 4 oxygens of **1** were accounted for in partial structures **A** and **B**, there were only 19 carbons in these substructures. Thus, in addition to units **A** and **B**, cumbiasin A (**1**) had one more quaternary carbon [δ_C 57.9, unit **C** in Figure 1]. Connections among unit **A** and the remaining six carbons (C-1, C-14, C-15, C-16, C-17, and C-20) encompassing substructures **B** and **C** were assigned on the basis of ^{1}H−^{13}C long-range correlations observed in the HMBC spectrum as follows (Table 1). The ^2J HMBC correlations from H-2 (δ_H 2.23), H-6 (δ_H 2.21), and H-9 (δ_H 2.34) to the low-field quaternary carbon at δ_C 57.9 (C-1) suggested multiple connectivities between units **A** and **C**, thereby constructing a substituted perhydroindene ring system (Figure 2). This was confirmed by complementary HMBC correlations between C-1 and H-3 (δ_H 2.18), H-7 (δ_H 1.72), and H-8β (δ_H 2.31). Moreover, ^3J HMBC correlations from H-2, H-6, and H-9 to the ketone carbonyl at δ_C 210.0 (C-14) suggested the connectivity between units **A** and **B** through C-1 in accordance with the relatively low-field ^{13}C resonance of C-1. Yet another pivotal connection between substructures **A** and **B** was deduced from ^2J HMBC correlations from H-2 to the ketone carbonyl at δ_C 212.8 (C-17). This allowed the connection between C-2 and C-17, thereby leading to a 1,4-cyclohexadione ring. Units **A** and **B** were linked through C-10 and C-16 by the observation of HMBC correlations between H-10 (δ_H 2.54) and C-15 (δ_C 82.7), C-16 (δ_C 77.2), and C-17 (δ_C 212.8) in a manner consistent with the complex tetracyclic array depicted in structure **1**. This key connectivity was supported by a weak but very diagnostic ^3J HMBC cross-peak between C-10 (δ_C 50.6) to 16-OH (δ_H 3.69). The aforementioned correlations were sufficient to propose the planar struc-

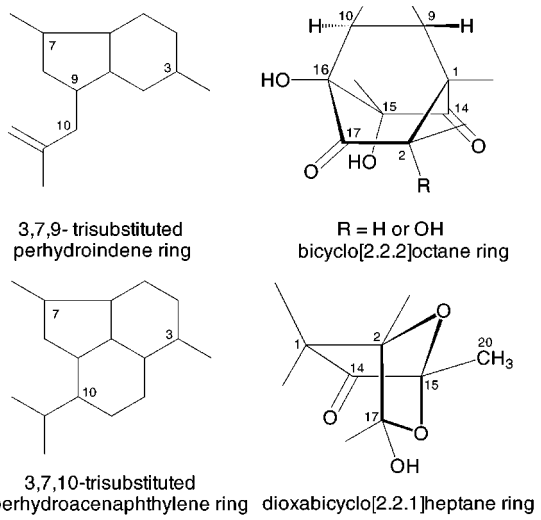


Figure 2. Polycyclic substructures embedded within the proposed structures of the cumbiasins A−C (**1**−**3**) with the names used in the text to identify them.

ture, **1**, including an unusual substructure consisting of a $C_8H_5O_4$ bicyclo[2.2.2]octane ring, which allowed the elimination of numerous inconsistent possibilities (Figure 2). Applying these combined NMR methods resulted in the assignment of all protons and carbons as listed in Table 1 and allowed the overall structure for cumbiasin A (**1**) to be assigned.

Since our repeated attempts to obtain suitable crystals for X-ray diffraction were unsuccessful, the relative stereochemistry of **1** was assigned primarily on the basis of NOESY NMR data and *J* values for the ^{1}H NMR spectrum (Table 1). The large coupling observed between H-2 and H-3 ($J_{2,3}$ = 10.7 Hz)[5] and between H-9 and H-10 ($J_{9,10}$ = 7.5 Hz)[6] suggested an anti orientation for these proton pairs. On the other hand, the lack of long-range coupling between H-2 and H-9 attests to their trans relationship since these protons, while part of the bicyclo-[2.2.2]octane framework, are not related by a planar W or zigzag pathway. Strong NOE cross-peaks between H-2 and Me-18, as well as significant through-space interactions between H-10 and both Me-20 (δ_H 1.48) and H-8α (δ_H 0.61), placed all of these groups on the (α) face. Furthermore, Me-19 exhibited intense NOEs with both H-8α and H-6 (δ_H 2.21) indicating that these protons are also on the same (α) face of the molecule. Most informative was a series of pronounced NOESY correlations among H-3 (δ_H 2.18), H-5β (δ_H 1.13), H-7 (δ_H 1.72), and H-9 (δ_H 2.34), consistent with their orientation on the opposite (top) face of the molecule. These correlations, all of which were consistent on the basis of a molecular modeling study,[7] were sufficient to establish the identity of the stereocenters at C-1 and C-16 as *S** and *R**, respectively. Thus, the overall relative stereochemistry for **1** was confidently assigned as 1*S**, 2*R**, 3*S**, 6*R**, 7*S**, 9*S**, 10*R**, 15*S**, and 16*R**. The intricate tetracyclic moiety present in **1** is not like that of any previously known terpenoid natural product. Therefore, cumbiasin A is the first member of an unprecedented class of rearranged diterpenes hereafter known as cumbianes.

Once the novel skeleton of **1** was elucidated, the structure elucidation of the natural derivative **2** proceeded in a smooth fashion with none of the NMR difficulties encountered for that of **1**. Cumbiasin B (**2**) is a colorless oil, $[\alpha]_D$ −29.0°. Its molecular formula was determined to be $C_{20}H_{28}O_5$ by HREI-MS (*m*/*z* 348.1952) and differs from that of **1** by the presence of one additional oxygen. The precise elemental composition of **2** was corroborated subsequently by HRFAB-MS *m*/*z* 371.1863 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_5Na$, 371.1834). The IR spectrum contained a strong hydroxyl stretching band at 3448 cm^{-1} in addition to a strong carbonyl band at 1734 cm^{-1} consistent with the presence of a 1,4-cyclohexadione moiety. The ^{13}C NMR in $CDCl_3$ (Table 2) contained 20 signals including seven quaternary carbons and four methyls, four methylene groups, of which one was a vinylic carbon (δ_C 114.4, C-12), and five methine carbons. The assignment of perhydroindene system resonances, as well as those of a $C_8H_5O_5$ bicyclo[2.2.2]octane ring, were entirely supported by 2D-NMR experiments and confirmed by comparison with data in **1** (Figure 2). The ^{1}H NMR and ^{13}C NMR chemical shifts of **2** (Table 2) were indeed very similar to those of **1**, suggesting that the only difference between **1** and **2** was the identity of the substituent at C-2. The only significant variations in the NMR data were the disappearance of the sharp doublet at δ_H 2.23 (*J* = 10.7 Hz) in the ^{1}H NMR spectrum of **1** due to H-2, the presence in **2** of three exchangeable protons, and the ^{13}C NMR chemical shift of C-2 (δ_C 52.9 versus 75.5) and C-18 (δ_C 22.0 versus 15.3), all of which were consistent with the presence of a hydroxyl at C-2 on the bottom face of the molecule (i.e., cis to Me-18) instead of a hydrogen atom. The ^3J HMBC couplings of C-2 (δ_C 75.5) with H-4α (δ_H 1.37), H-9 (δ_H 2.39) and H_3-18 (δ_H 1.27) supported this contention. The relative positions of the other functional groups were clearly supported by ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC results, which eventually allowed all protons and carbons in **2** to be assigned (Table 2). In the same way, the relative stereochemistry of the ring substituents in cumbiasin B (**2**) was determined to be the same as that found in **1** by a combination of NOESY, ^{1}H−^{1}H NMR coupling constant analysis (Table 2), and a molecular modeling study.[7]

The IR data for cumbiasin C (**3**), also a colorless oil, $[\alpha]_D$ +20.8°, indicated the presence of hydroxyl (3444 cm^{-1}) and carbonyl (1768, 1727 cm^{-1}) functionalities, the band at 1768 cm^{-1} strongly suggesting the presence of a strained cyclic ketone. HREI-MS data indicated a molecular ion consistent with a molecular formula of $C_{20}H_{26}O_5$. This molecular formula was validated by a LRFAB-MS *m*/*z* 353 $[M + Li]^+$ (calcd for $C_{20}H_{26}O_5Li$, 353) and differed from that of **2** by the loss of 2 Da. Inspection of the ^{1}H NMR spectral data of **3** (Table 3) showed a signal for one exchangeable proton at δ_H 5.12 (br s), two one-proton olefin signals at δ_H 5.03 and 4.80 (each br s), a deshielded one-proton signal at δ_H 3.23 (d, *J* = 13.5 Hz), two complex resonances at δ_H 2.29 (ddd, 1H, *J* = 6.5, 12.7, 13.5 Hz) and 1.66 (ddd, 1H, *J* = 2.7, 6.0, 6.0 Hz), and four methyl groups, suggestive of a polycyclic terpenoid structure. The ^{13}C NMR spectrum of cumbiasin C (Table 3) showed signals at δ_C 205.5 and 204.1 characteristic of two nonconjugated carbonyls, two olefin carbons at δ_C 138.9 and 116.4, two ketal-bearing carbons

(5) In compounds such as **1** containing six-membered rings fused to other structures, for conformations which do not depart appreciably from the chair form, $^3J_{axial-axial}$ is in the range of 8−13 Hz.

(6) In molecules rigidly held in the classical boat form (i.e., bicyclo-[2.2.2]octane), $^3J_{endo-exo}$ generally ranges from 5 to 7 Hz.

(7) The program Insight II (version 98.0) was employed for the molecular modeling study.

Table 2. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Cumbiasin B (2) in $CDCl_3$[a]

position	δ_H,mult, intgr, (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}HCOSY	NOESY	HMBC[c]
1		63.6 (s)			2-OH, H5α, H6, H7, H8*β*, H9
2		75.5 (s)			2-OH, H4α, H9, Me-18
3	2.04, m, 1H	33.6 (d)	H4α*β*, Me-18	H9, Me-18	H4α*β*, H5α*β*, Me-18
4α	1.37, m, 1H	28.9 (t)	H3, H4*β*, H5α*β*	Me-18	H3, H5*β*, Me-18
4*β*	1.52, m, 1H		H3, H4α, H5α*β*		
5α	1.94, m, 1H	30.8 (t)	H4α*β*, H5*β*, H6		H3, H4α, H6, H7
5*β*	1.17, m, 1H		H4α*β*, H5α, H6	H7, H9	
6	2.44, ddd, 1H (3.0, 7.1, 11.2)	39.1 (d)	H5α*β*, H7	Me-19	H4α, H8*β*, Me-19
7	1.73, m, 1H	40.3 (d)	H6, H8α*β*, Me-19	H5*β*, Me-19	H5*β*, H6, H8α*β*, H9, Me-19
8α	0.69, ddd, 1H (9.0, 12.5, 12.5)	40.7 (t)	H7, H8*β*, H9	H10	H6, H9, H10, Me-19
8*β*	2.27, ddd, 1H (7.3, 7.3, 12.8)		H7, H8α, H9		
9	2.39, ddd, 1H (7.1, 7.1, 12.4)	41.0 (d)	H8α*β*, H10	H3, H5*β*, Me-13	H6, H8α*β*, H10
10	2.62, d, 1H (7.1)	49.9 (d)	H9, H12α*β*	H8α, H12α, Me-20	H8α, H9, H12α*β*, Me-13, 16-OH
11		143.8 (s)			H9, H10, Me-13
12α	4.71, br s, 1H	114.4 (t)	H10, H12*β*, Me-13	H10, H12*β*	H10, Me-13
12*β*	4.91, t, 1H (1.3)		H10, H12α, Me-13	H12α, Me-13	
13	1.67, br s, 3H	21.9 (q)	H12α*β*	H9, H12*β*	H10, H12α*β*
14		208.8 (s)			H6, H9, Me-20
15		83.0 (s)			H10, Me-20
16		77.9 (s)			H10, Me-20
17		213.6 (s)			H10
18	1.27, d, 3H (6.7)	15.3 (q)	H3	H3, H4α	H3, H4α*β*
19	1.07, d, 3H (7.1)	20.9 (q)	H7	H6, H7	H6, H7, H8α
20	1.50, s, 3H	17.9 (q)		H10	
2-OH	2.95, br s, 1H				
15-OH	2.86, br s, 1H				
16-OH	3.58, br s, 1H				

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

Table 3. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOES,Y and HMBC Spectral Data of Cumbiasin C (3) in $CDCl_3$[a]

position	δ_H,mult, intgr, (*J* in Hz)	δ_C (mult)[b]	^{1}H−^{1}HCOSY	NOESY	HMBC[c]
1		55.0 (s)			H8*β*, H10
2		92.0 (s)			H4*β*, H9, 17-OH, Me-18
3	1.77, m, 1H	30.2 (d)	H4α*β*, Me-18	H9, Me-18	H4α*β*, H5α*β*, Me-18
4α	1.48, m, 1H	31.8 (t)	H3, H4*β*, H5α*β*		H3, H5α*β*, Me-18
4*β*	1.56, m, 1H		H3, H4α, H5α*β*		
5α	1.86, m, 1H	31.1 (t)	H4α*β*, H5*β*, H6		H3, H4α*β*, H6, H7
5*β*	1.27, m, 1H		H4α*β*, H5α, H6	H7, H9	
6	1.66, ddd, 1H (2.7, 6.0, 6.0)	46.3 (d)	H5α*β*, H7	Me-19	H4α*β*, H5α, H8*β*, Me-19
7	1.88, m, 1H	39.6 (d)	H6, H8α*β*, Me-19	H5*β*, Me-19	H6, H8α*β*, Me-19
8α	1.74, ddd, 1H (8.1, 12.3, 12.7)	35.0 (t)	H7, H8*β*, H9	Me-19	H7, H9, H10, Me-19
8*β*	1.95, ddd, 1H (6.5, 7.7, 12.3)		H7, H8α, H9		
9	2.29, ddd, 1H (6.5, 12.7, 13.5)	40.9 (d)	H8α*β*, H10	H3, H5*β*, Me-13	H8α*β*, H10
10	3.23, d, 1H (13.5)	53.8 (d)	H9, H12α*β*	H12α	H12α*β*, Me-13
11		138.9 (s)			H9, H10, H12α, Me-13
12α	4.80, br s, 1H	116.4 (t)	H10, H12*β*, Me-13	H10, H12*β*	H10, Me-13
12*β*	5.03, br s, 1H		H10, H12α, Me-13	H12α, Me-13	
13	1.71, br s, 3H	19.6 (q)	H12α*β*	H9, H12*β*	H10, H12α*β*
14		205.5 (s)			H6, H9, Me-20
15		103.3 (s)			Me-20
16		204.1 (s)			H9, H10, 17-OH
17		100.1 (s)			17-OH
18	1.30, d, 3H (6.6)	16.6 (q)	H3	H3	H3, H4α*β*
19	1.16, d, 3H (6.8)	20.4 (q)	H7	H6, H7, H8α	H7, H8α
20	1.69, s, 3H	12.1 (q)			
17-OH	5.12, br s, 1H				

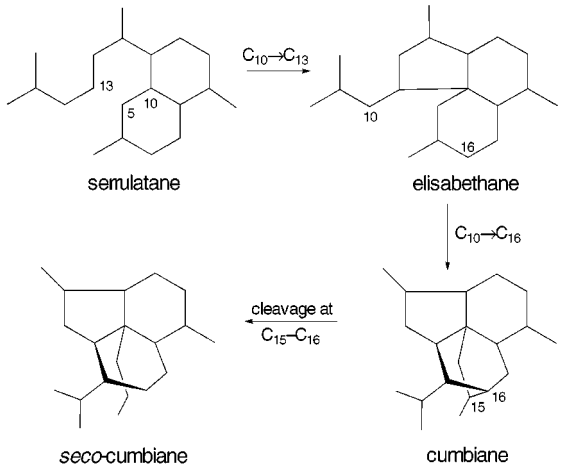
[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from an Attached Proton Test (APT) experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

at δ_C 103.3 and 100.1, a deshielded oxygenated carbon at δ_C 92.0, a quaternary carbon at δ_C 55.0, and 12 carbons between δ_C 12.1 and 53.8. The APT spectrum indicated that four CH_3, four CH_2, five CH, and seven quaternary carbons were present. Spectral evidence thus demanded that cumbiasin C was pentacyclic with one olefin and two carbonyl groups. Analysis of the overall NMR data and the ^{1}H−^{1}H COSY spectrum suggested the presence in **3** of essentially the same partial structures **A** and **C** present in cumbiasin A (**1**) (Figure 1). However, as with **2**, the ^{1}H NMR resonance ascribable to H-2 was absent in cumbiasin C (**3**). As in the case of **1** and **2**, substructures of **3** were established using a combination of homo- and heteronuclear NMR spectroscopic techniques. Key HMBC correlations from methyl protons were again used to connect these fragments, thereby allowing the construc-

tion of a substituted perhydroindene ring as that found in **1** and **2** (Figure 2). Further elaboration of the latter moiety into a $C_{12}H_{12}O_4$ perhydroacenaphthylene ring system (Figure 2) upon insertion of a C_2HO_3 bridge connected through C-2 and C-10 was entirely straightforward from the HMBC spectra (Table 3). Responses correlating with the ketone carbonyl at δ_C 204.1 (C-16) were couplings to H-9 (δ_H 2.29), H-10 (δ_H 3.23), and the pivotal 17-OH proton (δ_H 5.12). Further couplings between 17-OH and the carbon at δ_C 92.0 (C-2) and the ketal-bearing carbon at δ_C 100.1 (C-17) established unequivocally the linking of C-2 and C-16 through the C-17 ketal moiety in a fashion that is completely consistent with the presence of the proposed perhydroacenaphthylene ring. The connection between the ketone carbonyl at δ_C 205.5 (C-14) and the perhydroindene ring substructure through C-1 (δ_C 55.0) was deduced from $^3J_{CH}$ correlations from C-14 to H-6 (δ_H 1.66) and H-9 (δ_H 2.29). Furthermore, HMBC correlations from H_3-20 (δ_H 1.69) to C-14 and the ketal-bearing carbon at δ_C 103.3 (C-15) suggested the connectivity between C-14 and C-20 through C-15. The relative low-field resonance of C-2 (δ_C 92.0) indicated that this carbon was in a bridgehead position and that it was involved in an O-ether linkage with C-15. Having demonstrated spectroscopically the assembly of four out of five rings in **3**, all that remained to be assigned was the location and size of the remaining heterocycle. A more complex analysis, however, was required for the elucidation of the last unsaturation unit. To this end, we noticed that the only response correlated with C-15 was the $^2J_{CH}$ couplings to the C-20 methyl protons, which themselves showed no further couplings. Thus, the conspicuous absence of C−H long-range correlations from C-15 to either H-10 or the only hydroxyl proton in **3** revealed that, unlike in cumbiasins A (**1**) and B (**2**), there was no linkage between C-15 and C-16. From the analysis of these data we deduced the linking of C-15 with C-17 through an O-ether linkage and effectively established the structure of a C_5HO_4 dioxabicyclo[2.2.1]-heptane ring substructure (Figure 2) embedded in **3** and position it within the molecular framework in a manner consistent with the proposed structure of cumbiasin C.

With the overall structure of **3** defined, we next shifted our attention to deciphering its relative stereochemistry using a combination of NMR methods (NOESY and ^{1}H−^{1}H NMR coupling constants) coupled with a molecular modeling study.[7] The large coupling constant (13.5 Hz)[5] between H-9 and H-10 indicated a trans orientation for these protons. The isopropylene methyl protons (H_3-13) showed a pronounced NOESY correlation with H-9 (δ_H 2.29), which itself showed correlations with H-3 (δ_H 1.77), as well as with one of the methylene protons that correlated to C-5 (δ_H 1.27, H-5β). Similarly, a strong NOE interaction was observed between H-5β and H-7 (δ_H 1.88). These correlations placed H-3, H-5β, H-7, H-9, and the isopropylene group at C-10 within spatial proximity on the top face of the molecule. The configurations at C-6 and C-7 were defined as follows: H-6 (δ_H 1.66) showed a NOESY correlation with the C-19 methyl protons (δ_H 1.16), which were themselves placed in the (α) face of the molecule by a NOESY interaction with H-8α (δ_H 1.74). Fortunately, as a result of the rigid cagelike nature of the pentacyclic framework of cumbiasin C (**3**), the aforementioned correlations established the identity of the stereocenters at C-1, C-2, C-15, and C-17 as *S**. Therefore, the overall relative stereochemistry for **3** was assigned as 1*S**, 2*S**, 3*S**, 6*R**, 7*S**, 9*S**, 10*R**, 15*S**, and 17*S**. Compound **3** has also a logical structure from a biosynthetic viewpoint. Thus, cumbiasin B (**2**), which possesses five contiguous oxygen-bearing carbon atoms, could be envisioned as a precursor for cumbiasin C via enzyme-mediated 1,2-glycol oxidation giving an intermediate that then synchronously undergoes two ketalization steps. The stable dioxabicyclo[2.2.1]heptane moiety formed requires that the relative configuration at C-2 of the proposed biosynthetic precursor be *S** rather than *R** (i.e., 2-OH cis to Me-18). Unfortunately, scarcity of **2** precluded us from actually probing this biosynthetic proposal. A careful literature search of the substituted perhydroacenaphthylene ring system of cumbiasin C revealed no natural products composed of this specific tricyclic ring architecture. Therefore, we conclude that cumbiasin C (**3**) also represents a unique new class of diterpenes. Therefore, the name *seco*-cumbiane is proposed for this structurally unique carbon framework.

Scheme 1



Although not yet proven, the carbotetracyclic ring system of **1** and **2** appears to be produced by subsequent cyclization of a suitable elisabethane precursor (Scheme 1).[4a] Indeed, the isolation of both skeletal classes from the same specimen of *P. elisabethae* provides circumstantial support that the cumbiane ring system might be synthesized in vivo by subsequent cyclization of the elisabethane skeleton via [$C_{10} \rightarrow C_{16}$] bond formation. In an in vitro antituberculosis screen against *Mycobacterium tuberculosis* H_{37}Rv at 12.5 μg/mL, cumbiasin B (**2**) caused 17% inhibition in the primary screen. At a concentration of 6.25 μg/mL, cumbiasin A (**1**) coincidentally induced 17% growth inhibition of *M. tuberculosis*.

Experimental Section

General Experimental Procedures. Infrared spectra were recorded with a FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, DEPT, HMQC, and HMBC experiments were measured with a 500 MHz FT-NMR spectrometer. Column chromatography was performed on silica gel (35−75 mesh) or bonded C-18 silica gel (35−75 mesh). TLC analyses were carried out using glass precoated silica gel plates. All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the dry gorgonian MeOH−$CHCl_3$ extract.

Extraction and Isolation. The collection and extraction protocol followed has been described elsewhere.[4a] A portion of the hexane extract (50 g) of *P. elisabethae* was dissolved in a

small volume of toluene, filtered, and loaded onto a large Bio-Beads SX-3 column with toluene as eluant. Fractions were pooled on the basis of their TLC and NMR profile to yield four primary fractions, designated as I−IV. Fraction III (15.1 g) was separated into 18 subfractions by silica gel (270 g) column chromatography using 10% EtOAc in hexane. Subfraction III-13 (373 mg) was purified by column chromatography on silica gel (18 g) using 10% ethyl acetate in hexane to afford cumbiasin C (**3**) (9.0 mg; 1.13×10^{-2}% yield). Subfraction III-16 (612 mg) was purified by column chromatography on silica gel (25 g) using 15% ethyl acetate in hexane. A total of 17 fractions (A−Q) were obtained. Cumbiasin A (**1**) (7.6 mg; 9.53×10^{-3}% yield) was obtained pure after fraction III-16(J) (199.0 mg) was chromatographed successively over reversed-phase ODS silica gel (7.0 g) with 15% H_2O in MeOH followed by normal-phase silica gel (5.0 g) using 10% hexane in $CHCl_3$. Subfraction III-17 (588 mg) was chromatographed over silica gel (20.5 g) with 5% 2-propanol in hexane as eluant to yield seven fractions, designated as A−G. Fraction III-17(B) (163 mg) was purified further by silica gel (8.1 g) column chromatography with 10% MeOH in $CHCl_3$ to afford pure cumbiasin B (**2**) (6.7 mg; 8.40×10^{-3}% yield).

Cumbiasin A (1): colorless oil; $[\alpha]^{25}_D$ +6.7° (*c* 1.78, $CHCl_3$); IR (film) 3461, 3083, 1729, 1647, 1148, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 1); LRFAB-MS (3-NBA) *m/z* 333 $[M + 1]^+$ and *m/z* 355 $[M + Na]^+$; HREI-MS *m/z* $[M^+]$ calcd for $C_{20}H_{28}O_4$ 332.1987, found 332.1998 (14), 317.1794 (2, $C_{19}H_{25}O_4$), 314.1932 (2, $C_{20}H_{26}O_3$), 304.2105 (3, $C_{19}H_{28}O_3$), 261.1911 (40, $C_{17}H_{25}O_2$), 138.0685 (73, $C_8H_{10}O_2$).

Cumbiasin B (2): colorless oil; $[\alpha]^{25}_D$ −29.0° (*c* 1.56, $CHCl_3$); UV (MeOH) λ_{max} 208 nm (ϵ 7800); IR (film) 3448, 3078, 1734, 1649, 1127, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRFAB-MS (3-NBA) *m/z* $[M + Na]^+$ calcd for $C_{20}H_{28}O_5Na$ 371.1834, found 371.1863 (31); HREI-MS *m/z* $[M^+]$ calcd for $C_{20}H_{28}O_5$ 348.1937, found 348.1952 (1), 330.1907 (1, $C_{20}H_{26}O_4$), 302.1912 (8, $C_{19}H_{26}O_3$), 260.1721 (22, $C_{17}H_{24}O_2$), 259.1694 (100, $C_{17}H_{23}O_2$).

Cumbiasin C (3): colorless oil; $[\alpha]^{25}_D$ +20.8° (*c* 1.78, $CHCl_3$); UV (MeOH) λ_{max} 214 nm (ϵ 14100); IR (film) 3444, 3079, 1768, 1727, 1652, 1179, 1038, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 3); LRFAB-MS (3-NBA) *m/z* 353 $[M + Li]^+$; HREI-MS *m/z* $[M^+]$ calcd for $C_{20}H_{26}O_5$ 346.1780, found 346.1781 (5), 318.1840 (4, $C_{19}H_{26}O_4$), 303.1582 (4, $C_{18}H_{23}O_4$), 275.1645 (8, $C_{17}H_{23}O_3$), 259.1698 (15, $C_{17}H_{23}O_2$), 202.1719 (100, $C_{15}H_{22}$).

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Supporting Information Available: ^1H, ^{13}C NMR, and HMBC spectral data for compounds **1**−**3**, HMQC spectra for **1** and **2**, and NOESY spectra for **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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