Unusual Terpenes with Novel Carbon Skeletons from the West Indian Sea Whip *Pseudopterogorgia elisabethae* (Octocorallia)[1]

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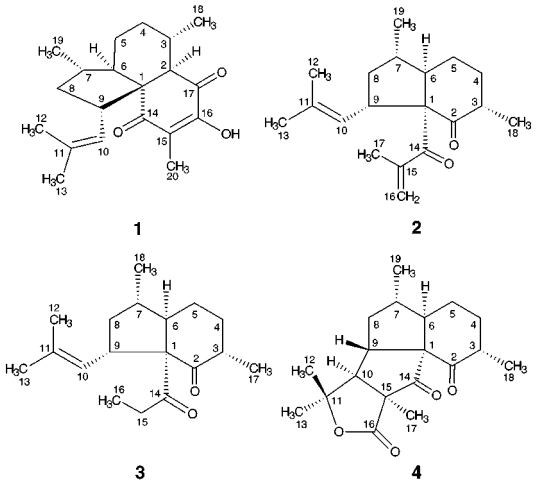
From the hexane solubles of the West Indian gorgonian *Pseudopterogorgia elisabethae* collected near San Andrés Island, Colombia, were isolated a marine diterpenoid, two *nor*-diterpenoids, and a *bisnor*-diterpenoid, all of which possess most unusual carbocyclic skeletons. The structures and relative configurations of novel metabolites elisabethins A−C (**1**−**3**) and elisabanolide (**4**) were elucidated by interpretation of overall spectral data, which included 2D NMR correlation methods; IR, UV, and accurate mass measurements (HREIMS); chemical reactions; and X-ray diffraction analyses. One of these, elisabethin B (**2**), showed significant differential antitumor activity, and compounds **3** and **4** have weak in vitro antituberculosis activity.

Introduction

As part of our continuing research program to assess the biomedical applications of marine organisms, we have focused considerable attention on the chemically rich gorgonians (sea fans, sea whips, and sea plumes) found in the reef habitats of the West Indian region.[3] Members of the genus *Pseudopterogorgia* are of particular interest since previous chemical investigations have revealed these animals to contain large quantities of highly bioactive terpenoid secondary metabolites.[4−6] The highly branched *Pseudopterogorgia elisabethae*, for instance, has been the subject of several chemical investigations, first by the Schmitz group[7] and more recently by the Fenical group.[8] The latter group has found that specimens of *P. elisabethae* collected from several locations throughout the Caribbean region contain pseudopterosins (and *seco*-pseudopterosins), an interesting class of natural products best characterized as diterpene−pentose−glycosides. Some pseudopterosins possess antiinflammatory and analgesic properties which exceed the potencies of existing drugs such as indomethacin.[9] During a recent underwater expedition to the Eastern Caribbean Sea, we collected a small sample of this animal (∼1 kg) in deep waters near San Andrés Island, Colombia. We were surprised to find that a subsequent extraction and fractionation of the crude extract of *P. elisabethae* from this location (see Experimental Section) resulted in the isolation of terpenoid metabolites which, unlike the pseudopterosins, lacked a sugar moiety. Moreover, a routine inspection of the ^{1}H and ^{13}C NMR data revealed that the majority of the compounds found in this specimen possessed distinctively different carbon frameworks quite unlike those already described for the aglycon portion of the pseudopterosins.[8] In this paper, we report the structures of four terpenoid compounds which are representatives of several new classes of previously undescribed natural products each possessing a novel carbon skeleton (Figure 1). The structures of compounds **1**−**4** were proposed on the basis of comprehensive spectral analyses, chemical transformations, and X-ray crystallographic analyses.



Results and Discussion

Isolation and Structure Elucidation. Freshly collected animals were sun-dried, stored frozen, and subsequently extracted with 50% MeOH/$CHCl_3$. Metabolites **1**−**4** were isolated by size-exclusion chromatography of the hexane extract using Bio-Beads SX-3 and were finally purified by successive normal-phase and/or reversed-phase silica gel column chromatography. Elisabethin B (**2**) was the major compound comprising almost 0.20% of

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(2) Graduate student sponsored by the NIH-MBRS Program of the University of Puerto Rico.

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds 1−4[a]

	Elisabethin A[b] (**1**)		Elisabethin B[c] (**2**)		Elisabethin C[d] (**3**)		Elisabanolide[e] (**4**)	
position	δ, mult, intgr (J in Hz)	^{13}C	δ, mult, intgr (J in Hz)	^{13}C	δ, mult, intgr (J in Hz)	^{13}C	δ, mult, intgr (J in Hz)	^{13}C
1		66.3, s		75.5, s		77.4, s		76.7, s
2	2.49, d, 1H (9.9)	53.4, d		213.2, s		213.6, s		209.2, s
3	2.39, br m, 1H	24.4, d	2.49, br m, 1H	46.7, d	2.45, br m, 1H	45.6, d	2.12, br m, 1H	45.4, d
4	1.21, br m, 1H	25.3, t	1.27, br m, 1H	29.9, t	1.46, br m, 1H	28.4, t	1.27, br m, 1H	29.1, t
4′	1.87, br m, 1H		1.67, br m, 1H		1.92, br m, 1H		1.60, br m, 1H	
5	1.26, br m, 1H	24.0, t	1.23, br m, 1H	19.1, t	1.46, br m, 1H	20.3, t	0.64, br m, 1H	27.2, t
5′	1.48, br m, 1H		2.14, br m, 1H		2.11, br m, 1H		1.90, br m, 1H	
6	2.52, br m, 1H	45.8, d	2.72, br m, 1H	51.3, d	2.54, br m, 1H	47.7, d	2.04, dt, 1H (6.9, 10.5)	53.6, d
7	1.77, br m, 1H	38.0, d	1.38, br m, 1H	34.2, d	1.54, br m, 1H	36.8, d	1.13, br m, 1H	42.8, d
8	1.14, br m, 1H	38.8, t	0.92, ddd, 1H (3.9, 5.4, 13.0)	40.2, t	0.88, br m, 1H	40.6, t	0.45, q, 1H (12.2)	44.3, t
8′	1.57, br m, 1H		2.20, br m, 1H		2.01, dt, 1H (7.9, 12.8)		1.70, ddd,1H (5.3, 7.2, 12.2)	
9	2.78, ddd, 1H (5.4, 5.7, 11.1)	47.2, d	4.22, ddd, 1H (5.4, 8.7, 11.1)	40.8, d	3.86, dt, 1H (7.7, 10.7)	42.1, d	3.18, ddd, 1H (2.1, 7.5, 9.8)	42.9, d
10	4.28, dd, 1H (1.2, 11.1)	127.3, d	4.90, br d, 1H (11.1)	126.2, d	4.72, br d, 1H (10.8)	125.9, d	1.85, d, 1H (2.1)	57.8, d
11		132.8, s		130.6, s		132.5, s		84.3, s
Me-12	1.43, d, 3H (1.2)	17.7, q	1.47, d, 3H (1.2)	17.7, q	1.72, d, 3H (0.7)	17.8, q	1.79, s, 3H	24.6, q
Me-13	1.50, d, 3H (1.2)	25.9, q	1.46, d, 3H (1.5)	25.6, q	1.62, br s, 3H	25.9, q	1.17, s, 3H	31.6, q
14		202.5, s		196.8, s		206.4, s		206.8, s
15		120.1, s		144.6, s	1.91, dq, 1H (7.1, 14.3)	34.2, t		62.2, s
15′					2.38, dq, 1H (7.1, 14.3)			
16		155.8, s	5.34, br d, 1H (1.2)	126.1, t	0.89, t, 3H (7.1)	7.3, q		172.0, s
16′			5.84, br s, 1H					
17		195.9, s	1.72, d, 3H (0.6)	19.0, q	1.08, d, 3H (7.5)	17.8, q	1.48, s, 3H	22.6, q
Me-18	1.09, d, 3H (6.3)	22.6, q	0.84, d, 3H (7.5)	16.3, q	0.97, d, 3H (6.5)	18.4, q	1.27, d, 3H (6.9)	18.8, q
Me-19	1.15, d, 3H (6.6)	18.9, q	0.77, d, 3H (6.6)	18.5, q			0.82, d, 3H (6.4)	17.3, q
Me-20	1.85, s, 3H	8.3, q						
OH	6.76, br s, 1H (exchangeable)							

[a] Assignments were aided by ^{1}H−^{1}H COSY, spin splitting patterns, analysis of J values, HMBC and HMQC experiments, numbers of attached protons as measured from DEPT spectra, and chemical shift values. The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals or, alternatively, the residual C_6H_6 (7.15 ppm) or C_6D_6 (128.0 ppm) signals. [b] Data recorded in $CDCl_3$ solution at 300 MHz. [c] Data recorded in C_6D_6 solution at 300 MHz. [d] Data recorded in $CDCl_3$ solution at 500 MHz. [e] Data recorded in C_6D_6 solution at 500 MHz.

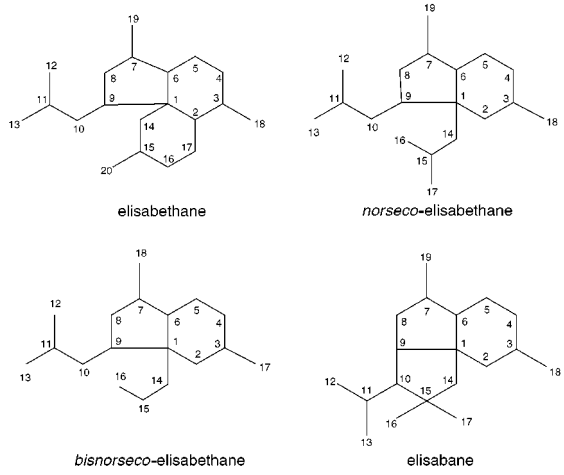

Figure 1. Novel terpenoid carbon skeletons isolated from *Pseudopterogorgia elisabethae* with proposed names and numbering systems.

the lipid extract, while elisabethin A (**1**), elisabethin C (**3**), and elisabanolide (**4**) represented 0.05%, 0.11%, and 0.12% of the hexane extract, respectively.

The minor metabolite, elisabethin A (**1**), was crystallized from $CHCl_3$ after successive normal-phase and reversed-phase column chromatography. Data from high-resolution mass and ^{13}C NMR spectroscopy (Table 1) established a molecular formula of $C_{20}H_{28}O_3$ for this metabolite, thus indicating 7 degrees of unsaturation. Because the ^{13}C NMR spectrum contained two carbonyl and four olefinic carbon resonances, the molecule was judged to be tricyclic. The ^{1}H NMR spectrum of compound **1** contained five methyl resonances [δ 1.85 (s, 3H), 1.50 (d, 3H, J = 1.2 Hz), 1.43 (d, 3H, J = 1.2 Hz), 1.15 (d, 3H, J = 6.6 Hz), 1.09 (d, 3H, J = 6.3 Hz)], which suggested that **1** possessed a diterpenoid carbon skeleton. The gross structure of elisabethin A (**1**) was deduced from analysis of one- and two-dimensional NMR spectra (Tables 1 and 2). Although **1** was soluble in CD_3OD and C_6D_6, the ^{1}H NMR signals in these solvents were broad and poorly dispersed. Consequently, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC NMR data were gathered in $CDCl_3$. A combination of HMQC and ^{1}H−^{1}H COSY spectral data (Table 2) allowed us to deduce partial structure **A**, whereas partial structure **B** was generated mainly from the ^{1}H and ^{13}C NMR, IR, UV, and HMBC spectral data (Figure 2). Although all 28 hydrogens and 3 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**, elisabethin A (**1**) had one more quaternary carbon (δ_C 66.3, unit **C** in Figure 2).

Four ^{13}C NMR resonances at δ 202.5 (s), 195.9 (s), 155.8 (s), and 120.1 (s), together with infrared absorptions

Table 2. $^1H-^1H$ COSY, NOESY, and HMBC Spectral Data of Elisabethin A (1) in $CDCl_3$

position	$^1H-^1H$COSY[a]	NOESY[a]	HMBC[b]
1			H-2, H-5, H-5′, H-6, H-8, H-8′, H-9
2	H-3	H-18	H-3, H-4, H-9, H-18
3	H-2, H-4, H-4′, H-18	H-4′, H-12, H-18	H-18
4	H-3, H-4′, H-5, H-5′	H-4′	H-18
4′	H-3, H-4, H-5, H-5′	H-3, H-4	
5	H-4, H-4′, H-5′	H-5′	H-7
5′	H-4, H-4′, H-5, H-6	H-5	
6	H-5′, H-7	H-19	H-2, H-7, H-8′, H-19
7	H-6, H-8, H-8′	H-8′, H-9, H-19	H-6, H-8′, H-9, H-19
8	H-7, H-8′, H-9	H-8′, H-19	H-7, H-9, H-10, H-19
8′	H-7, H-8, H-9	H-7, H-8, H-9	
9	H-8, H-8′, H-10	H-7, H-8′, H-12	H-2, H-6, H-8, H-10
10	H-9, H-12, H-13	H-13	H-8, H-9, H-12, H-13
11			H-9, H-12, H-13
Me-12	H-10	H-3, H-9	H-10, H-13
Me-13	H-10	H-10	H-10, H-12
14			H-2, H-6, H-9, H-20
15			H-20, -OH
16			H-20, -OH
17			H-2, H-20, -OH
Me-18	H-3	H-2, H-3	H-2, H-4
Me-19	H-7	H-6, H-7, H-8	H-6, H-7, H-8
Me-20			

[a] Spectra were recorded at 300 MHz at room temperature. [b] Spectrum recorded at 500 MHz. Protons correlated to carbon resonances in position column. Parameters were optimized for J_{CH} = 6 and 8 Hz.

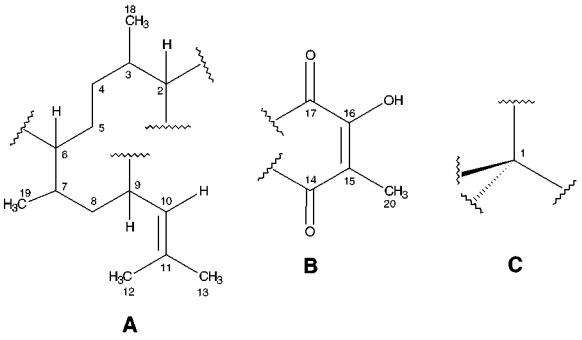

Figure 2. Partial structures leading to the structure of elisabethin A (**1**) generated from a combination of $^1H-^1H$ COSY, HMQC, and HMBC spectral data.

at 1688, 1652, and 1635 cm^{-1} and UV absorptions at 211 nm (ϵ 6500) and 278 nm (ϵ 6000), suggested that unit **B** was a fully substituted enedione system.[10] One substituent on this chromophore was proposed to be a vinyl methyl on the basis of a deshielded three-proton singlet resonance at δ 1.85.[10,11] A D_2O-exchangeable proton observed at δ 6.76 (br s, 1H) in the ^1H NMR spectrum of elisabethin A, together with a broadened infrared absorption at 3368 cm^{-1}, suggested that **1** was a monoalcohol, thus accounting for the remaining oxygen atom in the proposed molecular formula. A contention suggestive of the presence of a vinylic OH in substructure **B** came from HMBC correlations, which were optimized for 6- and 8-Hz couplings. In $CDCl_3$, the exchangeable hydroxyl proton at δ 6.76 showed a correlation to one of the carbonyl groups (δ 195.9, C17) and to both the oxygen-bearing vinylic carbon at δ 155.8 (C16) and the quaternary vinyl carbon at δ 120.1 (C15). These combined data indicated that the second substituent on the enedione portion in unit **B** was indeed a vinylic hydroxyl group.[10]

Connectivities from C2 to C10 in substructure **A**, including additional connectivities to two methyl groups attached to C3 and C7, were inferred from the $^1H-^1H$ COSY cross-peaks (Table 2). Further consideration of the ^{13}C NMR spectral data showed that elisabethin A possessed an additional trisubstituted olefin [δ 127.3 (d), 132.8 (s)] that was nonconjugated. Long-range correlations of a low-field resonance in the $^1H-^1H$ COSY spectrum of **1** [δ 4.28 (dd, 1H, J = 1.2, 11.1 Hz)] assigned to the olefinic proton (H-10) with two signals at δ 1.50 (d, 3H, J = 1.2 Hz, Me-13) and 1.43 (d, 3H, J = 1.2 Hz, Me-12) suggested that a 2-methyl-1-propenyl group was present in the molecule. The olefinic proton in substructure **A**, which is unusually shielded to δ 4.28, indicated that it must be forced into the π-cloud of substructure **B**.

Connectivities among the units in Figure 2 were determined by the correlations in the HMBC spectra: H-2/C1, H-2/C6, H-2/C9, H-2/C14, H-2/C17, H-6/C1, H-6/C9, H-6/C14, H-9/C1, H-9/C2, and H-9/C14, revealing all carbon connectivities of the complex tricyclic moiety of **1**. Because a δ 2.49 (H-2) methine proton in substructure **A**, which was a sharp doublet (J = 9.9 Hz) in the ^1H NMR spectrum, showed HMBC correlations to both the carbonyl carbons at δ 202.5 (C14) and δ 195.9 (C17) and the quaternary carbon at δ 66.3 (C1 in substructure **C**), it became evident that substructure **B** was part of a pentasubstituted cyclohexenedione ring system.[12] Confirmation of the substitution pattern of the latter system as well as the connectivities of all the ring systems found in **1** came from additional HMBC correlations found in Table 2 and the NOESY spectra, establishing the gross structure of elisabethin A.

A careful search of the literature did not reveal any known compounds of this skeletal type. While the spectral data of compound **1** were in full accord with the proposed structure for the molecule, an unambiguous proof of the structure was highly desirable. The structure of elisabethin A (**1**) was, therefore, confirmed by a single-crystal X-ray diffraction experiment which also yielded its relative stereochemistry. A computer-generated perspective drawing of the final X-ray model of **1**, less hydrogens, is shown in Figure 3. The absolute configuration of the molecule was not determined in the X-ray experiment. The novel carbon skeleton of elisabethin A (**1**), which we named elisabethane, represents a new class of diterpenes (Figure 1).

Elisabethin B (**2**), the major compound, was obtained as a colorless oil whose molecular formula was established as $C_{19}H_{28}O_2$ by HREIMS. The ^{13}C and DEPT NMR spectra (Table 1) showed 19 unique resonances: 5 quaternary, 5 methine, 4 methylene, and 5 methyl carbons. Chemical shift values further characterized two ketone carbonyls, four olefinic carbons, and one quater-

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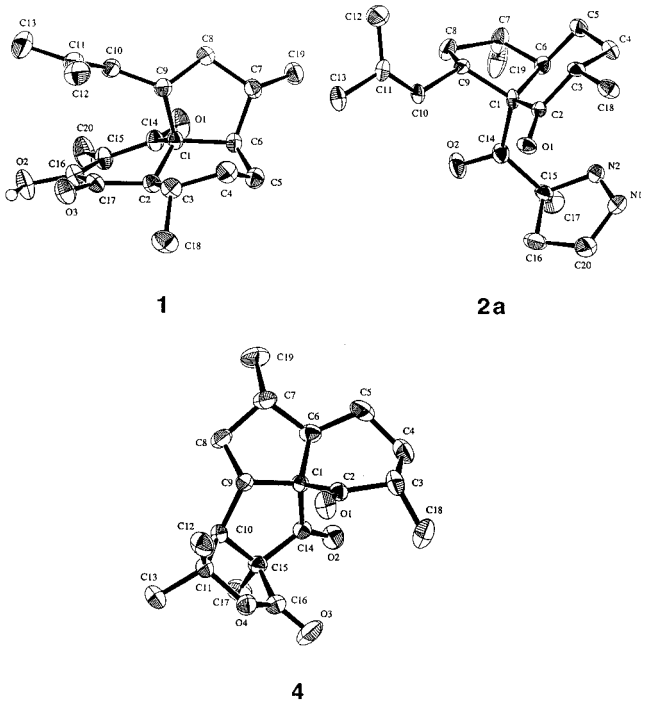
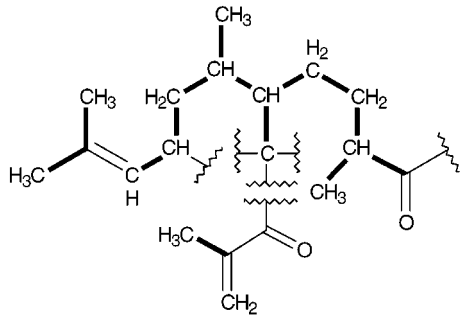


Figure 3. Computer-generated perspective drawings of the final X-ray crystallographic models of elisabethin A (**1**), pyrazoline derivative **2a**, and elisabanolide (**4**). Hydrogen atoms are omitted for clarity, and no absolute configuration is implied. The thermal ellipsoids are drawn at the 40% probability level.

nary aliphatic carbon. The IR spectrum suggested the absence of OH groups and confirmed the presence of a saturated ketone (1699 cm^{-1}) and a conjugated ketone (1661 cm^{-1}). The UV spectrum showed absorption at λ_{max} 225 nm (ϵ 2300) indicative of conjugation. The structural elucidation of elisabethin B (**2**) required 500-MHz 2D NMR analysis ($^1H-^1H$ COSY, HMQC, HMBC, NOESY, INADEQUATE) experiments employing both benzene-d_6 and $CDCl_3$ as solvents. The complete atom assignments for elisabethin B are recorded in Table 1.

Connectivities from C3 to C10 were inferred from the COSY cross-peaks, including correlations from H-3 to Me-18 and H-7 to Me-19. Allylic couplings between H-10 and both Me-12 at δ 1.47 (d, 3H, J = 1.2 Hz) and Me-13 at δ 1.46 (d, 3H, J = 1.5 Hz), led connectivity from C10 to C12 and C13. The assignment of the geminal vinylic methyls was established on the basis of the ^{13}C NMR chemical shifts and confirmed by observation of a NOESY correlation from H-10 to Me-13. The COSY spectrum also showed the signals at δ 5.34 (br d, 1H, J = 1.2 Hz, H-16) and δ 5.84 (br s, 1H, H-16′), ascribed to the methylene protons of a methacryloyl group, to be coupled to a vinyl methyl at δ 1.72 (d, 3H, J = 0.6 Hz, Me-17) which in turn showed no further couplings. These data allowed us to recognize this functionality as a terminal grouping. This was confirmed by high-resolution measurement of a $[M - C_4H_5O]^+$ peak at m/z = 219 (base peak). HMBC data provided the evidence to connect the quaternary carbon at δ 75.5 (C1) to the C6 and C9 methines, each of which was correlated with the C2 carbonyl group (δ 213.2). Thus, the connectivity from C6 and C9 to C2−CO via a C1 quaternary carbon was elucidated. That the quaternary carbon C1 was indeed vicinal to two carbonyl groups was judged from its low-field chemical shift value (δ 75.5). In the HMBC spectrum, the methine proton H-6 (δ 2.72) showed $^3J_{CH}$ coupling to the carbonyl carbon (δ 196.8, C14) within the methacryloyl side chain, thus allowing placement of this group at C1. Partial confirmation of the carbon connectivity network already established from the $^1H-^1H$ COSY and HMBC experiments was obtained directly from $^{13}C-^{13}C$ couplings. Since sample size was a limitation, many key carbon connectivities across the skeleton were not visible in the INADEQUATE experiment. The connectivity networks which helped to identify the unique carbon skeleton in elisabethin B (**2**) are illustrated in Scheme 1. The 2D INADEQUATE spectrum of **2** obtained in benzene-d_6 showed the cross-peaks of only 14 pairs of carbons. The correlations across the double bonds were of low intensity and were not visible in the plot.

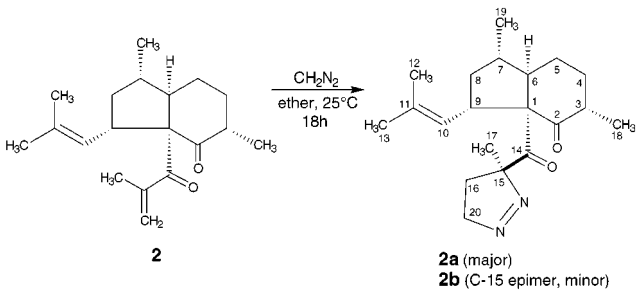
Scheme 1



The study of the relative stereochemistry of the chiral centers of elisabethin B (**2**) was initiated through extensive analysis of NOESY data in benzene-d_6 (Table S1 in Supporting Information). However, the relative positions and stereochemistries of the asymmetric centers in **2** could not be unambiguously assigned. As a possible solution to this problem we sought to prepare derivatives of **2** that were crystalline and suitable for X-ray diffraction studies. An attempted reaction of elisabethin B (**2**) with p-$BrC_6H_4NHNH_2$ failed to give the expected p-bromophenylhydrazone, and only unreacted **2** was recovered. Furthermore, because the spectral data from elisabethin B (**2**) established this metabolite to be a nonenolizable β-diketone, we attempted to remove the methacryloyl side chain at C1 through a retro-Claisen condensation reaction using 1.0 M KOH in MeOH. Surprisingly, even after a 26-h reflux, only unchanged starting material was recovered. Presumably, steric factors render backside approach at either C-2 or C-14 unfavorable. Treatment of elisabethin B (**2**) with diazomethane, on the other hand, gave a 2:1 mixture of pyrazoline derivatives **2a** and **2b** in good yield (Scheme 2).[13] Separation of these products by column chromatography led to major epimer **2a**, which fortunately crystallized, and an X-ray analysis provided the structure with relative stereochemistry. A computer-generated ORTEP drawing of the final X-ray model of pyrazoline **2a** is given in Figure 3. Hydrogens are omitted for clarity, and no information could be interpreted to assign the absolute configurations at the asymmetric centers. In general, bond distances and angles agreed well with generally accepted values. The cyclopentane and pyrazoline rings are essentially planar, and the cyclohexanone ring is in the half-chair conformation. From these data,

(13) Black, T. H. *Aldrichimica Acta* **1983**, *16*, 3−10.

Scheme 2



we inferred that in elisabethin B (**2**) the methyl groups at C3 and C7 and the hydrogen at the bridgehead (C6), as well as the 2-methyl-1-propenyl and methacryloyl side chains at C9 and C1, respectively, are all in a pseudoaxial conformation. Confirmation of this structure provided additional support for structure **3**, which was deduced exclusively from spectral analyses (vide infra). Elisabethin B (**2**) is without precedent in the natural products literature, and its novel carbon skeleton, named *norseco*-elisabethane, represents a new class of *nor*-diterpenes (Figure 1).

The molecular formula of elisabethin C (**3**) was determined to be $C_{18}H_{28}O_2$ by HREIMS (*m/z* 276.2078, Δ +1.1 mmu), which required 5 degrees of unsaturation. Because two signals due to olefinic carbons and two signals arising from carbonyl carbons were observed in the ^{13}C NMR spectrum (Table 1), **3** was shown to contain two rings. The double bonds were not conjugated since the UV showed only end absorption. The infrared spectrum of **3** confirmed the presence of two carbonyl groups (1716 and 1699 cm^{-1}), which accounted for all the oxygens in the proposed molecular formula. All 18 carbons appeared in the ^{13}C NMR spectrum, and a DEPT experiment indicated five methyls, four sp^3 methylenes, four sp^3 methines, one sp^2 methine, and four quaternary carbons (one sp^3 and three sp^2).

The gross structure of elisabethin C (**3**) was deduced from analysis of one- and two-dimensional NMR spectra (Table 1 and Table S2, Supporting Information). The ^1H and ^{13}C NMR spectra of **3** recorded in benzene-d_6 had many features in common with those of elisabethin B (**2**), and indeed $^1H-^1H$ COSY and HMQC experiments confirmed many of the same partial structures as determined for **2**, although the chemical shifts of the respective protons and carbons differed somewhat. The linkages from C3 through C10, including connectivities to Me-12, Me-13, Me-17, and Me-18, were unambiguously demonstrated in analogy to **2**. Nevertheless, distinctively different spectral features were observed in the ^1H NMR spectrum which ultimately indicated that the methacryloyl side chain in **2** had been replaced by a propanoyl group in elisabethin C (**3**). Critically, the ^1H NMR spectrum of **3** (in $CDCl_3$ solution) lacked the downfield singlets ascribed to the protons of a terminal conjugated enone system in **2** and instead revealed the presence of a terminal ethyl grouping [two signals at δ 1.91 (dq, 1H, J = 7.1, 14.3 Hz, H-15) and δ 2.38 (dq, 1H, J = 7.1, 14.3 Hz, H-15′) due to a methylene group coupled to the same proton signal at δ 0.89 (t, 3H, J = 7.1 Hz, Me-16)] (Table 1). Interestingly, the NMR signal for H-9 in **3** (and **2**) seemed quite far downfield for an allylic methine. This peculiarity suggests an unusual conformation where H-9 comes into some deshielding cone from a double bond or ketone. As in elisabethin B (**2**), the largest ion observed in the mass spectrum of **3** was *m/z* 219, consistent with the loss of a propanoyl radical ($CH_3CH_2CO^{\bullet}$) from the proposed molecular formula. Therefore, except for C1, the substitution pattern for the fused six-membered and five-membered rings in compound **3** was determined to be the same as that of elisabethin B (**2**).

NOEs were detected in $CDCl_3$ solution between H-6, Me-18, and H-15′ of elisabethin C (**3**), which indicated that these protons were all on the same (α) face of the molecule (Table S2, Supporting Information). Also, an NOE was observed between H-10 and Me-16; hence, the 2-methyl-1-propenyl side chain at C9 must be *cis* and parallel to the propanoyl group. An NOE observed between Me-18 and Me-16 provided additional evidence for the spatial proximity of these groups. On the other hand, no relevant NOEs were observed between Me-17 and protons on nearby positions. However, the similarities in coupling constant between these protons and their vicinal neighbors and the almost identical chemical shift values (in benzene-d_6 solution) indicated that Me-17 has the same relative stereochemistry in **3** as that observed in **2**. The name *bisnorseco*-elisabethane is proposed for the structurally unique carbon framework found in elisabethin C (**3**, Figure 1).

The molecular formula of elisabanolide (**4**) was determined to be $C_{19}H_{26}O_4$ by accurate mass measurement (318.1843, calcd 318.1831) plus ^1H and ^{13}C NMR data (Table 1). Therefore, like elisabethin B (**2**), elisabanolide was a *nor*-diterpene. As there were only resonances for three carbon−oxygen double bonds in the ^{13}C NMR spectrum of **4** [209.2 (s), 206.8 (s), and 172.0 (s)] and for no other multiple bonds, it was evident that the molecule must be tetracyclic. Elisabanolide (**4**) had an infrared spectrum with absorptions consistent with the presence of a γ-lactone and two ketone carbonyl groups (1770, 1750, and 1688 cm^{-1}, respectively). Because the carbonyl groups were not conjugated (the UV spectrum of **4** showed only end absorption), the absorption band at 1688 cm^{-1} was reasonably characteristic of a carbonyl functionality with its C−CO−C bond angle pushed outward above 120°. These functional groups accounted for all the oxygens in the proposed molecular formula. After association of all carbon signals with the corresponding signals for directly bonded protons via an HMQC experiment in benzene-d_6, $^1H-^1H$ COSY and HMBC spectral measurements were recorded (Table S3, Supporting Information). Furthermore, the HMQC and HMBC experiments allowed a complete assignment of the ^1H and ^{13}C NMR signals, as shown in Table 1.

The COSY spectrum allowed a continuous chain of $^1H-^1H$ coupling from H-3 to H-10 to be discerned and also revealed further couplings between H-3 and Me-18 and from H-7 to Me-19. The ^1H NMR spectrum of **4** differed considerably from that of *nor*-diterpene elisabethin B (**2**), but these differences were limited to the signals from protons C9, C10, C12, C13, C16, and C17. The remainder of the ^1H NMR spectrum of **4** could be matched with that of **2**, although some of the signals were slightly shifted. In the ^1H NMR spectrum, the H-9 proton signal was shifted from 4.22 to 3.18 ppm (relative to **2**) and was coupled, in addition to the C8 methylene protons, to a methine doublet signal at 1.85 ppm; the latter was thus assigned to H-10. Another signal, a 3H singlet at 1.48 ppm, was assigned to the Me-17 protons; the remaining methyl protons at 1.79 (s, 3H) and 1.17 (s, 3H) were ascribed, respectively, to Me-12 and Me-13 (in the COSY

spectrum Me-12 showed a clear long-range coupling to Me-13). In the ^{13}C NMR spectrum of **4**, the signals due to C1−C9, as well as those ascribable to C18 and C19, were quite similar to those of **2** and **3**, suggesting the same type of substituted cyclohexanone and cyclopentane rings. In particular, the chemical shift of C1 (δ 76.7) in **4** was almost the same as for **2** and **3**, which have similar substitution patterns on the adjacent carbons. However, the ^{13}C NMR spectrum of **4** revealed additional quaternary resonances at δ 62.2 and 84.3 in addition to a lactone carbonyl at δ 172.0, structural features not found in either **2** or **3**. There was also an additional methine signal at δ 57.8, and in the upfield portion of the spectrum, there were three methyl resonances at δ 22.6, 24.6, and 31.6. The annelation of two additional rings with substitution and oxidation patterns as depicted in structure **4** would account for these spectral differences.

In the HMBC experiment, the CH_3-15 protons showed heteronuclear couplings to C10 (δ 57.8), C14 (206.8), and C16 (172.0), and the H-10 signal correlated to C1 (δ 76.7), C8 (44.3), C9 (42.9), C14 (206.8), C15 (62.2), C16 (172.0), and C17 (22.6) (Table S3, Supporting Information). In a similar fashion, Me-13 was correlated to C10 (δ 57.8), C11 (84.3), and C12 (24.6). The chemical shift of C11 suggested an ester linkage at that point, and accordingly, the C16 lactone carbonyl showed HMBC correlations only to H-10 and Me-17. Interpretation of accurate mass spectral fragmentation data was used to confirm that the methyl groups Me-12, Me-13, and Me-17 were indeed situated in the γ-lactone ring in a manner consistent with structure **4** and that the lactone was in turn attached to a cyclopentanone ring system through C10 and C15 (see Supporting Information). Confirmation of the substitution pattern of the γ-lactone ring system, as well as the connectivities of all the ring systems found in **4** came from additional HMBC and NOESY correlations found in Table S3 (Supporting Information), which established the gross structure of elisabanolide.

The relative stereochemistries for all of the substituents on the fused cyclopentanone and γ-lactone rings in **4** were determined by analysis of proton−proton coupling constants and NOE experiments obtained in benzene-d_6 (Table S3, Supporting Information). An NOE between H-10 and Me-17 placed both of these groups on the α face and established the ring junction as *cis*. Another NOE between H-10 and Me-13 demonstrated that these protons are close to each other, placing Me-12 on the β face. Me-12 in turn exhibited an intense NOE with H-9, indicating that the latter proton is on the same (β) face of the molecule. On the other hand, the absence of significant through-space interactions between H-9 and H-10 and the small coupling observed between H-9 and H-10 ($J_{9,10} = 2.1$ Hz) suggested an antiparallel orientation of these protons. Since the ^{13}C NMR chemical shift values in benzene-d_6 of C1 (δ 76.7), C3 (45.4), C6 (53.6), C 18 (18.8), and C 19 (17.3) in elisabanolide (**4**) were in close agreement with the shift values of the corresponding carbons in elisabethin B (**2**) [C1 (δ 75.5), C3 (46.7), C6 (51.3), C18 (16.3), and C19 (18.5)], it seemed likely that the relative stereochemistry at those asymmetric centers in **4** remained unchanged. Fortunately, elisabanolide (**4**) gave good crystals from a mixture of hexane/CH_2Cl_2 by slow evaporation, allowing the structure to be confirmed by X-ray crystallography. A computer-generated drawing of elisabanolide is given in Figure 3. Hydrogens are omitted for clarity, and since the X-ray experiment did not define the absolute configuration, the enantiomer shown is an arbitrary choice. Thus the complete structure of elisabanolide with all relative stereochemistry is described by formula **4**. The complex tetracyclic ring system found in **4** is not like that of any previously known marine natural product.[6] Therefore, elisabanolide is the first member of an unprecedented class of marine natural products hereafter known as elisabanes (Figure 1).

Although the biosynthesis of metabolites **1**−**4** remains to be demonstrated, elisabethin A (**1**) can be considered as deriving from geranylgeranyl pyrophosphate via C1/C6, C1/C10, and C1/C13 cyclizations. On the other hand, the presence of bicyclic quinones such as **5** in several morphologically similar but chemically unique species of *Pseudopterogorgia*[8c] provides circumstantial support for a biosynthetic pathway in which the carbon skeleton of a *seco*-pseudopterosin is the precursor to the elisabethane skeleton (via C1/C13 cyclization; see Figure 4). Interestingly, the apparent mechanism by which diterpene **1** is produced is consistent in metabolites **2**−**4**, even though each has a completely different carbon skeleton. Elisabethin B (**2**) and elisabethin C (**3**), for instance, appear to be biosynthesized from **1** by the following reactions. The cyclohexanone ring of **2** and **3** is formed by oxidative cleavage of the bond between C2 and C17 in **1** after tautomerization. Loss of CO_2 followed by oxidation at C16 produces a key bicyclic β-ketocarboxylic acid (shown in brackets in Figure 4) which, after reduction and dehydration across C15 and C16, gives **2** or alternatively, upon decarboxylation, yields **3**. In turn, elisabanolide (**4**) appears derived from subsequent condensation and annelation of the proposed bicyclic β-ketocarboxylic acid precursor. Thus, the cyclopentanone ring of **4** is formed by attack of the anion of the active methine at C15 (adjacent to the C14 and C16 carbonyls) on the C10 position of an epoxide group between C10 and C11. Intramolecular esterification of the carboxylic acid functionality with a tertiary carbinol at C11 gives the five-membered lactone moiety in **4**.

Biological Activity. Biological screening of elisabethin B (**2**) in the National Cancer Institute's (NCI) 60-cell-line tumor panel indicated significant in vitro cancer cell cytotoxicity with concentrations of 10^{-5} M eliciting significant differential responses at the GI_{50} level from all the renal, CNS, and leukemia cancer cell lines.[14] One cell line, however, [i.e., NCI-H226 (non-small-cell lung cancer)] was substantially more sensitive than the average. In an in vitro antituberculosis screen (against *Mycobacterium tuberculosis* H37Rv) at 12.5 μg/mL, elisabethin C (**3**) and elisabanolide (**4**) caused, respectively, 42% and 39% inhibition in the primary screen.[15] We also examined elisabethin B (**2**) as a possible topical antiinflammatory agent using an in vivo assay.[16] However, doses of **2** below 0.3 mM/ear were not significantly effective against bee venom PLA_2, arachidonic acid (AA), and croton oil-induced inflammation in mouse ears.

(14) (a) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubenstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092. (b) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

(15) Restogi, N.; Goh, K. S.; Van Ginkel, S. Z.; Wright, E. L.; Barrow, W. W. *Res. Microbiol.* **1996**, *147*, 97−105.

(16) (a) Tubaro, A.; Dri, P.; Melato, M.; Mulas, G.; Bianchi, P.; Del Negro, P.; Della Loggia, R. *Agents Actions* **1986**, *19*, 371−373. (b) Tubaro, A.; Dri, P.; Delbello, G.; Zilli, C.; Della Loggia, R. *Agents Actions*, **1985**, *17*, 347−349.

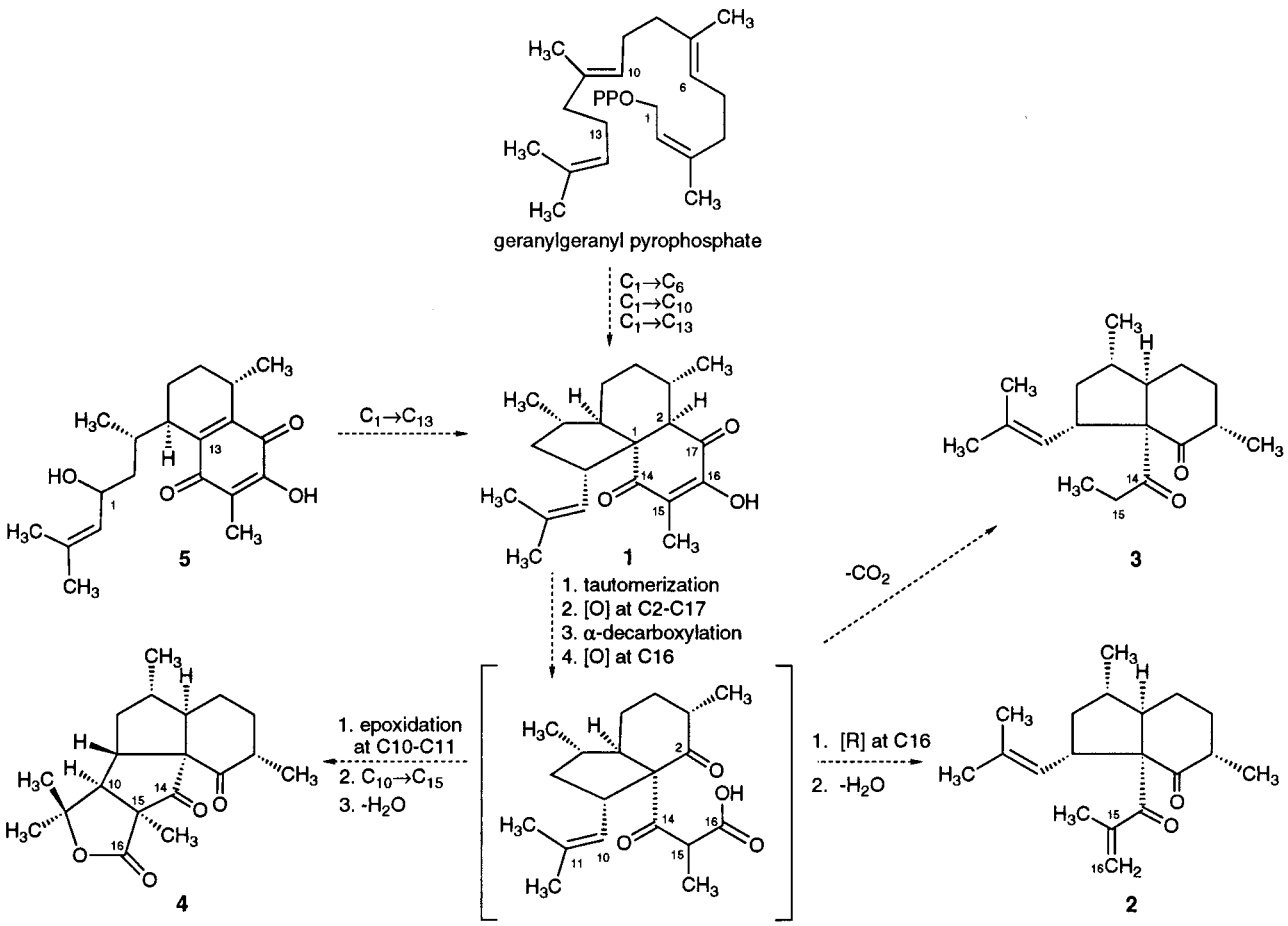


Figure 4. Possible biosynthetic pathways of elisabethins A−C (**1**−**3**) and elisabanolide (**4**). A bicyclic *p*-benzoquinone **5** (isolated from a Bahamian *Pseudopterogorgia* species) related to the aglycon portion of the *seco*-pseudopterosins, was included in the biosynthetic scheme due to its close analogy to the *P. elisabethae*-derived terpenes described here.

Compounds **1**, **3**, and **4** proved inactive in the NCI's test for agents active against the human inmunodeficiency virus (HIV).[17] Additional studies to assess the biological properties of compounds **1**−**4** are currently underway.

Experimental Section

General Experimental Procedures. Infrared spectra were recorded on a FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, DEPT, HMQC, HMBC, and 2D-INADEQUATE experiments were measured on either a 500-MHz or a 300-MHz FT-NMR spectrometer. Column chromatography was performed on silica gel (35−75 mesh), and 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. Diazomethane was prepared in-house according to literature procedures.[18] Diazald and *p*-bromophenylhydrazine were purchased from Aldrich Chemical Co. The percentage yield of each compound is based on the weight of the dry gorgonian specimen.

Collection and Extraction of *P. elisabethae*. The Caribbean sea whip *P. elisabethae* was collected by hand using SCUBA at depths of 80−100 ft during May 1996, off San Andrés Island, Colombia. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The gorgonian was sun-dried and kept frozen prior to its extraction. The dry animal (1.0 kg) was blended with MeOH−$CHCl_3$ (1:1) (11 × 1 L), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (284 g). After the crude extract was partitioned between hexane and H_2O, the resulting extract was concentrated in vacuo to yield 178 g of an oil, a portion of which (50 g) was dissolved in a small volume of toluene, filtered, and loaded onto a large Bio-Beads SX-3 column with toluene as eluent. 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 into 18 subfractions by silica gel (270 g) column chromatography using 10% EtOAc in hexane as eluent. Fraction 3.1 (3.18 g), which was subsequently purified by column chromatography [silica gel (150 g) with 2% EtOAc in hexane], afforded elisabethin B (**2**) (89 mg; % yield, 3.2×10^{-2}). Elisabethin C (**3**) (55.9 mg; % yield, 2.0×10^{-2}) was obtained pure after purification of fraction 3.4 (62.5 mg) by column chromatography on silica gel (6.0 g) using a mixture of 2% EtOAc in hexane as eluent. Fraction 3.7 (140.8 mg) was chromatographed successively over silica gel (6.0 g) with 2% EtOAc in hexane and then ODS silica gel (2.0 g) with 2% H_2O in MeOH to yield pure elisabethin A (**1**) (25.0 mg; % yield, 8.9×10^{-3}). Fraction 3.17 (588.1 mg) was chromatographed successively over silica gel (20.5 g) with 5% 2-propanol in hexane and then with 10% EtOAc in $CHCl_3$ to yield pure elisabanolide (**4**) (62 mg; % yield, 2.2×10^{-2}).

Elisabethin A (1): crystalline solid; $[\alpha]^{25}_D$ +133.0° (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} 211 nm (ϵ 6500), 278 nm (ϵ 6000); IR (film) 3368, 2954, 2925, 2873, 1688, 1652, 1635, 1454, 1381, 1322, 1301, 1281, 1163, 1091, 820, 793 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m/z* [M$^+$] calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2048 (12), 288.2082 (10, $C_{19}H_{28}O_2$), 286.1884 (11, $C_{19}H_{26}O_2$), 234.1227 (10, $C_{14}H_{18}O_3$), 208.0978 (19, $C_{15}H_{12}O$), 207.0932 (100, $C_{12}H_{15}O_3$), 179.1111 (31, $C_{11}H_{15}O_2$), 91.0701 (18, $C_4H_{11}O_2$).

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(18) Hudlicky, M. *J. Org. Chem.* **1980**, *45*, 5377.

Single-Crystal X-ray Diffraction Analysis of Elisabethin A. Crystallization of elisabethin A by slow evaporation from $CHCl_3$ yielded colorless crystals of excellent quality. A specimen of 0.40 × 0.32 × 32.00 mm was selected for the analysis. X-ray diffraction data were collected on a Siemens SMART CCD system at 26 ± 1 °C to a maximum 2θ of 54.1°, using Mo Kα radiation (λ = 0.710 69 Å). Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice constants of a = 9.5998(5), b = 13.0726(7), and c = 14.1281(7) Å. The systematic extinctions, crystal density (d_{calc} = 1.185 g/cm^3), and optical activity indicated space group $P2_12_12_1$ in the asymmetric unit (Z = 4) of composition $C_{20}H_{28}O_3$ with formula weight of 316.44. Of the 9583 reflections measured, 2100 were unique (R_{int} = 0.018); equivalent reflections were merged. The crystallographic residual was R = 5.5% (R_w = 7.4%) for the observed reflections. The structure, which was solved by direct methods (SIR92) and completed by successive Fourier calculations, was refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. H08 was refined with a fixed isotropic thermal parameter and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corporation.[19] Neutral atom scattering factors were taken from *International Tables for X-ray Crystallography*.[20]

Elisabethin B (2): colorless oil; $[\alpha]^{25}_D$ −99.0° (*c* 1.1, $CHCl_3$); UV (CH_3OH) λ_{max} 225 nm (ϵ 2300); IR (film) 2952, 2925, 2869, 1699, 1661, 1633, 1455, 1376, 1308, 1238, 1130, 1090, 1052, 1027, 940, 843 cm^{-1}; ^{1}H NMR (C_6D_6, 300 MHz) and ^{13}C NMR (C_6D_6, 75 MHz) (see Table 1); LRFAB-MS *m*/*z* $[M + H]^+$ calcd for $C_{19}H_{29}O_2$ 289.4, found 289.4; HREI-MS *m*/*z* $[M^+]$ calcd for $C_{19}H_{28}O_2$ 288.2089, found 288.2086 (13), 273.1860 (8, $C_{18}H_{25}O_2$), 260.2085 (38, $C_{18}H_{28}O$), 245.1900 (21, $C_{17}H_{25}O$), 233.1574 (11, $C_{15}H_{21}O_2$), 219.1749 (100, $C_{15}H_{23}O$), 217.1589 (73, $C_{15}H_{21}O$), 203.1422 (81, $C_{14}H_{19}O$), 179.1077 (27, $C_{11}H_{15}O_2$), 161.1028 (52, $C_{11}H_{13}O$), 151.1119 (20, $C_{10}H_{15}O$), 145.0996 (31, $C_{11}H_{13}$), 119.0869 (46, C_9H_{11}), 105.0668 (42, C_8H_9), 95 (43, C_7H_{11}), 91.0493 (55, C_7H_7), 69.0330 (52, C_4H_5O).

Elisabethin C (3): colorless oil; $[\alpha]^{25}_D$ −31.2° (*c* 0.5, $CHCl_3$); IR (film) 2955, 2922, 2851, 1716, 1699, 1457, 1375, 1261, 1164, 1094, 1024, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 1); HREI-MS *m*/*z* $[M^+]$ calcd for $C_{18}H_{28}O_2$ 276.2089, found 276.2078 (0.4), 245.1520 (5, $C_{16}H_{21}O_2$), 219.1748 (100, $C_{15}H_{23}O$), 203.1411 (9, $C_{14}H_{19}O$), 167.1084 (16, $C_{10}H_{15}O_2$), 165.1291 (15, $C_{11}H_{17}O$), 119.0863 (14, C_9H_{11}), 109.1036 (23, C_8H_{13}), 91.0537 (25, C_7H_7), 81.0689 (29, C_6H_9), 73.0647 (81, C_4H_9O), 69.0699 (39, C_5H_9), 57.0356 (32, C_3H_5O).

Elisabanolide (4): colorless oil; $[\alpha]^{25}_D$ −39.0° (*c* 0.4, $CHCl_3$); IR (film) 2976, 2952, 2937, 2922, 2867, 2858, 1770, 1750, 1688, 1456, 1374, 1262, 1233, 1193, 1166, 1147, 1079, 967, 948, 939, 904 cm^{-1}; ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) (see Table 1); HREI-MS *m*/*z* $[M^+]$ calcd for $C_{19}H_{26}O_4$ 318.1831, found 318.1843 (50), 303.1574 (22, $C_{18}H_{23}O_4$), 290.1885 (100, $C_{18}H_{26}O_3$), 272.1775 (16, $C_{18}H_{24}O_2$), 246.1996 (32, $C_{17}H_{26}O$), 235.1330 (25, $C_{14}H_{19}O_3$), 232.1466 (16, $C_{15}H_{20}O_2$), 231.1754 (40, $C_{16}H_{23}O$), 217.1583 (40, $C_{15}H_{21}O$), 203.1431 (37, $C_{14}H_{19}O$), 191.1432 (93, $C_{13}H_{19}O$), 175.1129 (23.8, $C_{12}H_{15}O$), 164.1217 (92, $C_{11}H_{16}O$), 149.0949 (23, $C_{10}H_{13}O$), 135.1182 (45, $C_{10}H_{15}$), 127.0768 (35, $C_7H_{11}O_2$), 109.1007 (35, C_8H_{13}), 91.0543 (34, C_7H_7), 79.0568 (38, C_6H_7).

Single-Crystal X-ray Diffraction Analysis of Elisabanolide. Crystallization of elisabanolide by slow evaporation from a mixture of hexane/CH_2Cl_2 yielded colorless crystals of excellent quality. A specimen of 0.61 × 0.51 × 0.44 mm selected for the analysis was mounted on a glass fiber. X-ray diffraction data were collected on a Siemens SMART CCD system at 24 ± 1 °C to a maximum 2θ of 54.1°, using Mo Kα radiation (λ= 0.710 69 Å). Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice constants of a = 6.4303(4), b = 35.957(2), and c = 7.5980(4) Å. The systematic extinctions, crystal density (d_{calc} = 1.204 g/cm^3), and optical activity indicated space group $P2_12_12_1$ in the asymmetric unit (Z = 4) of composition $C_{19}H_{26}O_4$ with formula weight of 318.41. Of the 9303 reflections measured, 3596 were unique (R_{int} = 0.017); equivalent reflections were merged. The crystallographic residual was R = 3.7% (R_w = 4.6%) for the observed reflections. The structure, which was solved by direct methods (SIR92) and completed by successive Fourier calculations, was refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. H08 was refined with a fixed isotropic thermal parameter, and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corporation.[19] Neutral atom scattering factors were taken from *International Tables for X-ray Crystallography*.[20]

Synthesis of Pyrazoline Derivatives 2a and 2b. A solution of diazomethane in ether[18] (15 mL) was added at once to a solution of elisabethin B (**2**) (9.7 mg, 0.034 mmol) in CH_2Cl_2 (10 mL). After 18 h of stirring at 25 °C, the solvent was removed and the mixture of pyrazolines (10.7 mg, obtained in a 2:1 ratio) was separated over a short column of silica gel using a mixture of 10% EtOAc in hexane as eluent. The main epimer **2a** crystallized in a test tube (7.1 mg) after slow evaporation of the solvent. Major epimer **2a**: yellowish crystals; $[\alpha]^{25}_D$ +19.2° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 210 nm (ϵ 5600), 330 nm (ϵ 600); IR (film) 2957, 2930, 2893, 2871, 1707, 1681, 1555, 1450, 1374, 1263, 1161, 1070, 1039, 980, 884, 811 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.56 (br d, 1H, J = 9.1 Hz, H-10), 4.50 (ddd, 1H, J = 4.0, 10.6, 18.1 Hz, H-20), 4.27 (dt, 1H, J = 8.6, 18.1 Hz, H-20′), 3.24 (dd, 1H, J = 6.8, 9.9 Hz, H-6), 3.12 (dt, 1H, J = 8.8, 11.7 Hz, H-9), 2.26 (br m, 1H, H-3), 2.16 (br m, 2H, H-8, H-5), 2.02 (br m, 3H, H-4, H-7, H-16), 1.87 (br m, 1H, H-5′), 1.69 (br m, 2H, H-4′, H-8′), 1.65 (s, 3H, Me-17), 1.50 (br m, 1H, H-16′), 1.44 (s, 3H, Me-13), 1.42 (s, 3H, Me-12), 1.04 (d, 3H, J = 7.1 Hz, Me-19), 0.95 (d, 3H, J = 6.5 Hz, Me-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 212.3 (s, C-2), 209.2 (s, C-14), 133.7 (s, C-11), 122.9 (d, C-10), 99.2 (s, C-15), 75.5 (t, C-20), 75.1 (s, C-1), 52.0 (d, C-6), 46.3 (d, C-9), 41.8 (d, C-3), 39.9 (d, C-7), 39.5 (t, C-8), 31.8 (t, C-5), 31.6 (t, C-4), 29.9 (t, C-16), 26.4 (q, C-13), 26.1 (q, C-17), 22.5 (q, C-19), 17.8 (q, C-12), 15.2 (q, C-18); LRFAB-MS *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}N_2O_2$ 331.2, found 331.2; HREI-MS *m*/*z* $[M - H_2O]^+$ calculated for $C_{20}H_{28}N_2O$ 312.2201, found 312.2202 (20), 284.2244 (17, $C_{19}H_{28}N_2$), 269.2004 (7, $C_{18}H_{25}N_2$), 241.1707 (17, $C_{16}H_{21}N_2$), 228.1629 (92, $C_{15}H_{20}N_2$), 219.1747 (87, $C_{15}H_{23}O$), 213.1388 (25, $C_{14}H_{17}N_2$), 203.1194 (24, $C_{12}H_{15}N_2O$), 201.1037 (29, $C_{12}H_{13}N_2O$), 193.1236 (56, $C_{12}H_{17}O_2$), 186.1157 (49, $C_{12}H_{14}N_2$), 165.0920 (54, $C_{10}H_{13}O_2$), 160.1005 (11, $C_{10}H_{12}N_2$), 145.1013 (17, $C_{11}H_{13}$), 109.1022 (25, C_8H_{13}), 91.0548 (40, C_7H_7), 83.0502 (79, C_5H_7O), 69.0703 (22, C_5H_9), 55.0541 (100, no matches found). Minor epimer **2b**: yellowish oil, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.58 (br d, 1H, J = 9.3 Hz, H-10), 4.66 (ddd, 1H, J = 3.3, 9.9, 18.0 Hz, H-20), 4.20 (dt, 1H, J = 9.0, 18.0 Hz, H-20′), 3.14 (dt, 1H, J = 8.2, 12.0 Hz, H-9), 2.32 (br m, 1H), 2.07 (br m, 1H), 1.93 (br m, 1H), 1.86 (br m, 2H), 1.71 (br d, 3H, J = 1.2 Hz, Me-13), 1.68−1.42 (broad envelope, 4H), 1.50 (br d, 3H, J = 1.5 Hz, Me-12), 1.38 (s, 3H, Me-17), 1.23 (d, 3H, J = 6.6 Hz, Me-19), 1.05 (dd, 1H, J = 4.4, 6.9 Hz), 0.93 (dd, 1H, J = 6.4, 11.8 Hz), 0.82 (d, 3H, J = 6.9 Hz, Me-18).

(19) Molecular Structure Corporation (1985, 1992). *TEXSAN. Crystal Structure Analysis Package*. MSC, 3200 Research Forest Drive, The Woodlands, TX, 77381.

(20) Cromer, D. T.; Waber, J. T. *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, U.K. 1974; Vol. IV, Tables 2.3.1 and 2.2A.

Single-Crystal X-ray Diffraction Analysis of Pyrazoline Derivative 2a. Crystals of pyrazoline derivative **2a** were grown from a hexane/ethyl acetate solution by slow evaporation. The compound crystallized as colorless prisms with dimensions 0.65 × 0.41 × 0.41 mm. X-ray diffraction data were collected on an Enraf−Nonius diffractometer (CAD4) at 22 ± 1 °C to a maximum 2θ of 55.9°, using graphite monochromated Mo Kα radiation (λ = 0.710 69 Å). Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice constants of a = 9.442(1), b = 10.8697(9), and c = 19.079(2) Å. The systematic extinctions, crystal density (d_{calc} = 1.121 g/cm^3), and optical activity indicated space group $P2_12_12_1$ in the asymmetric unit (Z = 4) of composition $C_{20}H_{30}N_2O_2$ with formula weight of 330.47. Of the 3049 reflections measured, 2270 were unique (R_{int} = 0.026); equivalent reflections were merged. The crystallographic residual was R = 7.3% (R_w = 10.3%) for the observed reflections. The structure, which was solved by direct methods (SIR92) and completed by successive Fourier calculations, was refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. H08 was refined with a fixed isotropic thermal parameter, and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corporation.[19] Neutral atom scattering factors were taken from *International Tables for X-ray Crystallography*.[20]

Attempted Retro-Claisen Condensation of Elisabethin B (2). To a solution of elisabethin B (20 mg, 0.07 mmol) in MeOH (5 mL) was added 1.0 M methanolic KOH (0.1 mL). After 18 h of stirring at 25 °C, a mild reflux was initiated and then maintained for 26 h. The reaction mixture was cooled to room temperature, quenched to neutrality with a few drops of 0.5 N HCl, and concentrated. Aqueous workup including extraction with $CHCl_3$ (3 × 15 mL) led to the recovery of 17.4 mg of unreacted **2** after concentration and storage under high vacuum.

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Supporting Information Available: Description of the X-ray crystal structures data including large ORTEP drawings, tables of intramolecular distances, torsion angles, positional parameters, and intramolecular bond angles for elisabethin A (**1**), pyrazoline derivative **2a**, and elisabanolide (**4**); ^{1}H and ^{13}C NMR spectra and ^{1}H−^{1}H COSY, NOESY, and interpretation of the HREI mass spectral data for **4**; and HMBC spectral data for compounds **2**−**4** (Tables S1-S3) (51 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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