Novel Terpenoids from the West Indian Sea Whip *Pseudopterogorgia elisabethae* (Bayer). Elisapterosins A and B: Rearranged Diterpenes Possessing an Unprecedented Cagelike Framework[1]

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Four diterpenes and a *nor*-diterpenoid, all of which possess unusual carbocyclic skeletons, were isolated from the hexane solubles of the West Indian gorgonian *Pseudopterogorgia elisabethae*. The structures and relative configurations of novel metabolites elisabethin D (**2**), elisabethin D acetate (**3**), 3-*epi*-elisabanolide (**5**), elisapterosin A (**6**), and elisapterosin B (**7**) 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), chemical reactions, and X-ray diffraction analyses. The tetracyclic carbon skeleton of the elisapterosins is undescribed and constitutes a new class of C_{20} rearranged diterpenes. Elisapterosin B displays strong in vitro anti-tuberculosis activity.

In the past decade, a large number of structurally interesting diterpenoids, such as cembranes, pseudopteranes, gersolanes, amphilectanes, and serrulatanes, have been discovered from gorgonian corals of the genus *Pseudopterogorgia* (family Gorgoniidae, order Gorgonacea, phylum Cnidaria).[3] In the course of our search for novel bioactive substances for potential biomedical uses from Caribbean gorgonians (sea whips, sea feathers, sea fans, and sea plumes) *Pseudopterogorgia* diterpenes continue to be the target of our investigations. Species of the genus *Pseudopterogorgia* are of current interest as sources of unique metabolites because of the interesting biological activities (e.g., cytotoxic, ichthyotoxic, neurotoxic, antiinflammatory, analgesic, and antibacterial) associated with the compounds isolated from them.[4] Our first investigation on the chemical constituents of a specimen of *Pseudopterogorgia elisabethae* (Bayer) collected in San Andrés Island, Colombia, resulted in the isolation of four structurally interesting diterpenoids, among which elisabethin A (**1**) and elisabanolide (**4**), possessing the uncommon elisabethane and elisabane carbon skeletons, respectively, stood out as having the most intricate structures.[5] Subsequent chemical investigations of this gorgonian specimen also revealed the presence of rearranged terpenoids of the *nor*-sandresane skeletal class[6] in addition to amphilectane- and serrulatane-based diterpenes.[7−9] In connection with our continuing interest in the development of new agents for the treatment of tuberculosis, a new investigation on the secondary metabolites of *P. elisabethae* has now led to the isolation and structural elucidation of two novel diterpenes, elisapterosins A (**6**) and B (**7**), possessing the unprecedented cagelike elisapterane carbon skeleton, in addition to two new elisabethins, elisabethin D (**2**) and elisabethin D acetate (**3**), and a new elisabane *nor*-diterpene, 3-*epi*-elisabanolide (**5**). The structures and relative stereochemistry of compounds **2**, **3**, and **5**−**7** were established by the use of a series of 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) and chemical transformations. The structure and relative configuration of elisabethin D (**2**), 3-*epi*-elisabanolide (**5**), and

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(9) We have also detected traces of pseudopterosins in our gorgonian specimen. The presence of these amphilectane-based diterpene glycosides is usually indicative of *P. elisabethae* and thus constitutes a useful chemotaxonomic marker.

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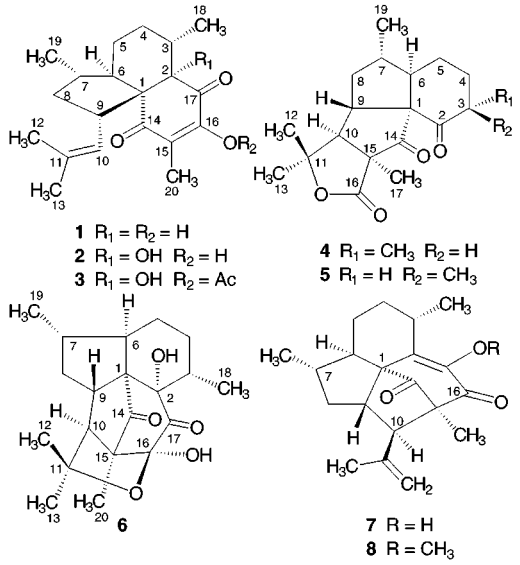
elisapterosin B (**7**) were further confirmed by single-crystal X-ray diffraction analysis. Elisapterosin B was found to possess strong in vitro inhibitorial activity against *Mycobacterium tuberculosis* H37Rv.



Elisabethin D (**2**) was obtained as colorless block crystals, $[\alpha]^{25}_D$ +6.4° (*c* 1.25, $CHCl_3$). HREI-MS established a molecular formula of $C_{20}H_{28}O_4$, and its IR spectrum suggested the presence of hydroxyl (ν_{max} 3513 and 3260 cm^{-1}), carbonyl (ν_{max}1703 and 1622 cm^{-1}), and olefin (ν_{max}1645 cm^{-1}) functionalities. The ^1H NMR spectrum of **2** showed two exchangeable singlets at δ 6.47 and 2.18 (1H each), indicating the presence of two hydroxyl groups. Other features of the spectrum included a doublet at δ 4.28 (J = 10.8 Hz) and two broad methyl doublets at δ 1.53 and 1.44 (J = 1. 2 Hz each) indicative of an isobutenyl group, one sharp three-proton singlet at δ 1.91 ascribable to a vinyl methyl group, two sharp three-proton doublets at δ 1.12 (J = 6.9 Hz) and 1.17 (J = 6.6 Hz), indicating two secondary methyl groups, and a one-proton doublet of doublets of doublets at δ 2.83 (J = 5.4, 10.8, 12.3 Hz), suggesting a hydrogen atom on a carbon bearing an olefin. The ^{13}C NMR spectrum exhibited 20 signals ($5CH_3$, $3CH_2$, 5CH, and 7C) whose chemical shift values and multiplicity confirmed the presence of a fully substituted enedione system [δ 200.2 (s), 192.8 (s), 153.3 (s), 121.9 (s)], a trisubstituted olefin [δ 127.1 (d), 132.5 (s)], a deshielded quaternary carbon [δ 69.9 (s)], a shielded methyl group [δ 8.6 (q)], and one oxygen-bearing carbon [δ 78.3 (s)]. Spectral evidence thus demanded that elisabethin D (**2**) was tricyclic with two olefins and two carbonyl groups. Thus, in common with compound **1**, it appeared that elisabethin D contained an elisabethane skeleton.[5] The structure of **2** was determined and the relative stereochemistry assigned from a single-crystal X-ray diffraction analysis (Figure 1). A combination of a series of 2D NMR methods ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY) resulted in the unambiguous assignment of all protons and carbons as listed in Table 1.

Elisabethin D acetate (**3**) was isolated as a colorless oil. Its molecular weight was determined at *m/z* 374, corresponding to the molecular formula $C_{22}H_{30}O_5$ (HREI-MS *m/z* 374.2061). All spectral data for **3** were very similar to those of **2**, except for the presence of the acetyl

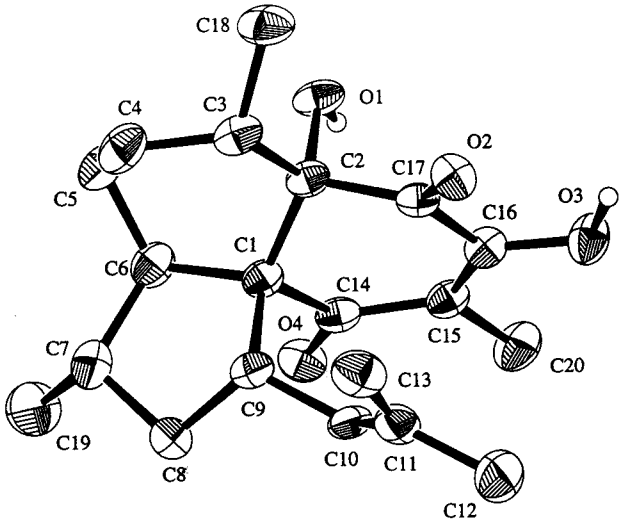


Figure 1. Computer-generated perspective drawing of the final X-ray crystallographic model of elisabethin D (**2**). The thermal ellipsoids are drawn at the 40% probability level, and no information could be interpreted to assign the absolute configurations at the asymmetric centers. The hydrogen atoms are omitted for clarity.

group at C-16. The presence in the ^1H NMR spectrum of **3** of a broad hydroxyl singlet at δ 2.68, when considered with the absence of a sharp exchangeable singlet near δ 6.50 that could be ascribed to a vinyl hydroxyl (as in the case of **1** and **2**), and the low-field chemical shift value of the acetyl methyl group in **3** (δ 2.27) supported the placement of the acetate group at C-16. This assignment was further confirmed by the straightforward acetylation of **2** at 25 °C with acetic anhydride in pyridine to give **3**. As in the case of **2**, comprehensive NMR data allowed all protons and carbons to be assigned (see the Experimental Section).

3-*epi*-Elisabanolide (**5**), isolated as a minor crystalline compound, $[\alpha]^{25}_D$ −4.0° (*c* 1.5, $CHCl_3$), $C_{19}H_{26}O_4$ by HREI-MS, showed 19 resonances in its ^{13}C NMR spectrum, which according to a DEPT spectrum were associated with five methyl, three methylene, five methine, and six quaternary carbons. The absence of a significant UV absorption in compound **5**, together with ^{13}C NMR signals due to quaternary carbons at δ 206.2 (s, C-2), 205.6 (s, C-14), 172.1 (s, C-16), 84.7 (s, C-11), 76.2 (s, C-1), and 62.3 (s, C-15), three broad three-proton ^1H NMR singlets at δ 1.63 (Me-17), 1.62 (Me-12), and 1.53 (Me-13), a one-proton doublet of doublets at δ 3.52 (J = 6.3, 6.9 Hz, H-9), and a one-proton doublet at δ 2.20 (J = 6.9 Hz, H-10), indicated that **5** possessed the same elisabane skeleton found in elisabanolide (**4**).[5] Information gleaned from $^1H-^1H$ COSY, NOESY, HMQC, and HMBC spectra (see Table 1) led to formulation of structure **5** for 3-*epi*-elisabanolide, and this was confirmed by X-ray crystallographic analysis. A perspective ORTEP plot of **5** is shown in Figure 2. The X-ray experiment did not define the absolute configuration. All ^1H and ^{13}C NMR spectral assignments and a summary of HMBC correlations are presented in Table 1.

The structure elucidation of elisapterosin A (**6**) commenced when the molecular formula of $C_{20}H_{28}O_5$ was established on the basis of HREI-MS data and overall NMR information. This result was eventually validated by HRFAB-MS *m/z* 349.1998 $[M + H]^+$ (calcd for $C_{20}H_{29}O_5$ 349.2015). In addition to a strong IR absorption at 3467

Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and HMBC Spectral Data for Elisabethin D and 3-*epi*-Elisabanolide in $CDCl_3$[a]

	elisabethin D (**2**)			3-*epi*-elisabanolide (**5**)		
position	δ_H, mult, intgr, (*J*, Hz)	δ_C (mult)	HMBC[b]	δ_H, mult, intgr, (*J*, Hz)	δ_C (mult)	HMBC[b]
1		69.9 (s)	H8α*β*, H9		76.2 (s)	H6, H8*β*, H9
2		78.3 (s)	H9, H18		206.2 (s)	H3, H6, H9, H18
3	2.52, m, 1H	28.0 (d)	H4α*β*, H5α*β*, H18	3.04, m, 1H	43.2 (d)	H18
4α	1.26, m, 1H	25.1 (t)	H3, H5α*β*, H6, H18	1.48, dd, 1H (3.6, 13.2)	31.0 (t)	H3, H18
β	1.97, m, 1H			2.00, m, 1H		
5α	1.97, m, 1H	25.1 (t)	H3, H4α*β*, H6	2.12, dt, 1H (4.8, 13.8)	21.7 (t)	H3, H4α
β	1.26, m, 1H			1.66, m, 1H		
6	2.59, m, 1H	43.7 (d)	H7, H8*β*, H19	1.92, m, 1H	55.1 (d)	H5*β*, H8*β*, H9, H19
7	1.73, m, 1H	38.5 (d)	H5α, H8α*β*	1.71, m, 1H	37.1 (d)	H5α*β*, H8α*β*
8α	1.05, q, 1H (12.0)	39.9 (t)	H7, H9, H19	1.07, ddd, 1H (6.3, 9.9, 12.9)	42.1 (t)	H10, H19
β	1.63, m, 1H			2.40, dt, 1H (8.4, 12.9)		
9	2.83, ddd, 1H (5.4, 10.8, 12.3)	45.3 (d)	H6, H8α	3.52, dd, 1H (6.3, 6.9)	40.3 (d)	H8α, H10
10	4.28, d, 1H (10.8)	127.1 (d)	H8α*β*, H9, H12, H13	2.20, d, 1H (6.9)	60.6 (d)	H8α*β*, H9, H12, H13, H17
11		132.5 (s)	H9, H12, H13		84.7 (s)	H9, H12, H13
12	1.44, br d, 3H (1.2)	17.6 (q)	H10, H13	1.62, br s, 3H	24.4 (q)	H10, H13
13	1.53, br d, 3H (1.2)	26.0 (q)	H10, H12	1.53, br s, 3H	30.5 (q)	H10, H12
14		200.2 (s)	H6, H9, H20		205.6 (s)	H6, H9, H17
15		121.9 (s)	16-OH		62.3 (s)	H10, H17
16		153.3 (s)	H20, 16-OH		172.1 (s)	H10, H17
17		192.8 (s)	16-OH	1.63, br s, 3H	23.9 (q)	H10
18	1.12, d, 3H (6.9)	17.0 (q)	H3, H4α	1.00, d, 3H (6.6)	14.6 (q)	H3
19	1.17, d, 3H (6.6)	18.9 (q)	H7, H8α	0.96, d, 3H (6.6)	17.9 (q)	H8α
20	1.91, s, 3H	8.6 (q)				
2-OH	2.18, br s, 1H					
16-OH	6.47, s, 1H					

[a] Assignments were aided by ^{1}H−^{1}H COSY, spin splitting patterns, HMBC, HMQC, and NOESY experiments, numbers of attached protons, and chemical shift values. The δ values are in parts per million and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. [b] Protons correlated to carbon resonances in the position column. Parameters were optimized for $^{2,3}J_{CH} = 6$ and 8 Hz.

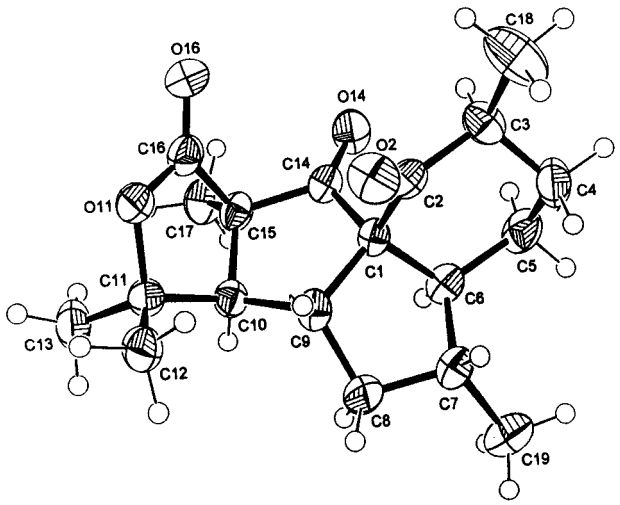


Figure 2. ORTEP view of 3-*epi*-elisabanolide (**5**). The thermal ellipsoids are drawn at the 40% probability level, and no information could be interpreted to assign the absolute configurations at the asymmetric centers.

cm^{-1} indicative of hydroxyl groups, intense bands at 1746 and 1722 cm^{-1} indicated two ketone functions, one of which (1746 cm^{-1}) was tentatively assigned to a cyclopentanone moiety on the basis of the ^{13}C NMR signal at δ 212.9 (s). The ^{1}H NMR spectrum, initially obtained in $CDCl_3$ (Table 2), showed two exchangeable protons, five methyl groups (two secondary and three quaternary), and eleven complex proton resonances between δ 0.6 and δ 3.2, suggestive of a polycyclic terpenoid structure. The ^{13}C NMR spectrum of **6** contained signals for all 20 carbons, including the following: two ketone carbonyls (δ 212.9, 209.9), three oxygenated quaternary carbons, one of which was accounted for by a hemiacetal functionality (δ 106.0, 84.6, 81.7), three methylenes (δ 42.0, 27.6, 23.3), five methine carbons (δ 60.5, 43.6, 42.8, 39.6, 38.3), and five methyl groups (δ 30.6, 25.9, 18.8, 18.1, 14.7). The remaining signals were ascribable to two quaternary sp^3 carbons (δ 62.1, 61.3). The absence of UV absorption, when considered with the lack of olefinic carbons, indicated that the two carbonyls in compound **6** were present as two nonconjugated ketones. The remaining five degrees of unsaturation required that the molecule possess four carbocyclic rings and a cyclic hemiacetal.

The molecular structure of **6** was defined on the basis of a standard series of one- and two-dimensional NMR experiments, which included ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC. Detailed analyses of these spectra led to assignments of proton connectivities for the three spin systems shown in Figure 3. Of the three spin systems, only one system (**A**) was present in all the previous compounds (**2**−**5**), and system **B** was common only to elisabanolides **4** and **5**. Only one system (**C**) was different from those already encountered in metabolites **2**−**5**. Thus, the structure elucidation of the first two systems proceeded in a smooth fashion with none of the difficulties found for that of partial structure **C**. This substructure contained a three-carbon fragment −C(CH_3)−C(OH)−O bearing a hydroxyl substituent assigned as cis to a vicinal methyl group on the basis of NOESY correlations from Me-20 to H-10 and 16-OH.

Fragments **A** and **B** were connected through C-11, on the basis of HMBC correlations (HMBC experiments optimized for $^{2,3}J_{CH} = 6$ and 8 Hz) of that carbon to protons at δ 3.17 (H-9), 1.99 (H-10), 1.48 (Me-13), and 1.40 (Me-12). An intense NOESY correlation supporting this connection was that between Me-12 and H-9. HMBC correlations from Me-20 to C-10, C-15, and C-16, along with those observed to C-15 from H-9 and 16-OH, provided partial connection between fragments **A** and **C**. Similarly, NOESY correlations between Me-20 and Me-

Table 2. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Elisapterosin A (6) in $CDCl_3$[a]

position	δ_H, mult, intgr, (*J*, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		61.3 (s)			H3, H6, H8α*β*, H10, 2-OH
2		81.7 (s)			H3, H4α*β*, H6, H18
3	2.12, m, 1H	38.3 (d)	H4α*β*, H18	H18	H18
4α	1.26, m, 1H	27.6 (t)	H3, H4*β*, H5α*β*		H3, H18
β	2.04, m, 1H		H3, H4α, H5α*β*		
5α	1.81, m, 1H	23.3 (t)	H4α*β*, H5*β*, H6		
β	1.53, m, 1H		H4α*β*, H5α, H6		
6	2.03, m, 1H	43.6 (d)	H5α*β*, H7	H19	H5α*β*, H8*β*, H19
7	1.68, m, 1H	42.8 (d)	H6, H8α*β*, H19	H8*β*, H9,[d] H19	H6, H8α*β*, H19
8α	0.65, q, 1H (12.1)	42.0 (t)	H7, H8*β*, H9	H10, H19	H6, H10, H19
β	1.94, m, 1H		H7, H8α, H9	H7, H9	
9	3.17, ddd, 1H (3.3, 6.9, 9.9)	39.6 (d)	H8α*β*, H10	H7,[d] H8*β*, H12	H8α, H10
10	1.99, d, 1H (3.3)	60.5 (d)	H9	H8α, H13, H20[d]	H8α, H12, H13, H20
11		84.6 (s)			H9, H10, H12, H13
12	1.40, s, 3H	25.9 (q)		H9, H13	H13
13	1.48, s, 3H	30.6 (q)		H10, H12, 16-OH, H20[d]	H10, H12
14		212.9 (s)			H6, H9, H20
15		62.1 (s)			H20, 16-OH
16		106.0 (s)			H10, H20, 16-OH
17		209.9 (s)			
18	0.62, d, 3H (6.9)	18.8 (q)	H3	H3	H3, H4α*β*
19	0.99, d, 3H (6.6)	18.1 (q)	H7	H6, H7, H8α	H6, H8α
20	1.42, s, 3H	14.7 (q)		H10,[d] H13,[d] 16-OH	H10
2-OH	2.79, br s, 1H				
16-OH	4.03, br s, 1H			H13, H20	

[a] Chemical shift values are in parts per million relative to TMS. Spectra were recorded at room temperature. [b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences. [c] Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz. [d] This strong NOE was observed clearly in Bz-d_6 solution.

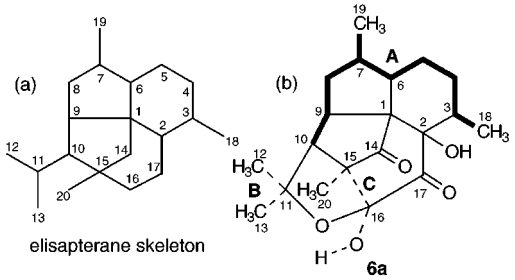


Figure 3. (a) Novel terpenoid carbon skeleton of elisapterosins A and B with the proposed name and numbering system. (b) Partial structures of elisapterosin A. The bold lines in **6a** indicate discrete spin systems identified by NMR experiments. The broken lines indicate spin systems separated by more than three bonds and identified by their long-range ^{1}H−^{1}H COSY or NOESY correlations. The letter adjacent to each system is used to identify an individual spin system discussed in the text.

13, detected clearly only when the NOESY spectrum of **6** was recorded in benzene-d_6,[10] established the spatial proximities of these protons. This dipolar ^{1}H−^{1}H connectivity together with that observed between Me-13 and 16-OH (in $CDCl_3$ solution) provided useful, albeit limited, connectivity between fragments **B** and **C** through C-10 and across the cyclic hemiacetal link, respectively. The correlation between Me-20 and the cyclopentanone carbonyl at δ 212.9 (C-14), which was further correlated to the protons on C-6 (δ 2.03) and C-9 (δ 3.17), provided the connection among C-6, C-9, and C-14 through C-1 (δ 61.3). Additional correlations supporting this connection were those from C-1 to H-3, H-6, H-8α*β*, H-10, and 2-OH. Finally, the tertiary carbinol at δ 81.7 was linked to fragment **A** at C-3 on the basis of the observed correlations from C-2 to H-3 and Me-18, and at C-6 through C-1 on the basis of the correlation between H-6 and C-2. What remained to be assigned was the carbonyl carbon at δ 209.9 (C-17), which had to be linked to fragments **A** and **C** through C-2 and C-16 to close the remaining carbocycle. No correlation between H-3 and C-17 was observed in the HMBC experiments with **6**; however, the rather low field chemical shifts of C-2 (δ 81.7) and C-16 (δ 106.0) supported the bridging of carbons C-2 and C-16 through C-17. Confirmation of the structures of the three spin units as well as the sequencing was provided by HREI-MS fragmentation data (see the Supporting Information). Applying these combined NMR and HREI-MS methods resulted in the unambiguous assignment of all protons and carbons as listed in Table 2 and allowed the complete planar structure for **6** to be assigned.

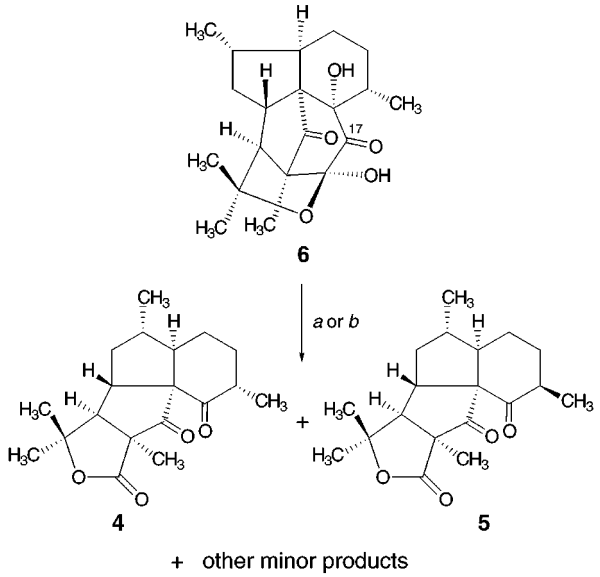
The relative configurations of the stereocenters in the tetracyclic nucleus of elisapterosin A (i.e., C-1, C-2, C-3, C-6, C-7, C-9, C-10, C-15, and C-16) were assigned using a combination of NMR methods (NOESY and ^{1}H−^{1}H NMR coupling constants) coupled with NMR spectral comparisons and molecular modeling studies. The C-10/C-15/C-16 ring fusions were assigned as cis on the basis of strong NOESY correlations among H-10, Me-20, and the hemiacetal hydroxyl proton (16-OH). Similarly, NOESY correlations among Me-13, H-10, and the C-16 hydroxyl proton established the spatial proximities of these protons on the bottom face of the molecule. Most informative was a pronounced NOESY correlation between H-9 and Me-12, consistent with their cis orientation on the opposite (top) face of the molecule. Both C-9 and C-10 having the *S** configuration was supported by the small 3.3 Hz coupling constant observed between H-9 and H-10, consistent with the trans orientation shown in structure **6** ($^3J_{trans}$ is always notably smaller than $^3J_{cis}$ in five-membered rings, which cannot deviate appreciably from planarity).[11] The configurations at C-6 and C-7 were defined as follows: the C-6 methine proton showed a NOESY correlation with the C-19 methyl protons, which

(10) The ^{1}H NMR chemical shifts for these protons in benzene-d_6 were δ 1.26 (Me-20) and 1.54 (Me-13).

were themselves placed in the α face of the molecule by a NOESY interaction with H-8α. Likewise, H-7 showed a NOESY correlation to H-9 as well as H-8*β*. Consequently, we have assigned the configurations of C-6 and C-7 as *R** and *S**, respectively. No correlation between H-9 and Me-18 was observed in the NOESY experiments with **6**; thus, the stereocenter at C-3, which lies isolated from the remainder of the molecule, was defined as *S** (i.e., trans to H-9). Comparison of the ^{13}C NMR spectra obtained for elisabathin D (**2**), elisabathin D acetate (**3**), and elisapterosin A (**6**) suggested that the methyl group at C-3 (Me-18) and the C-2 hydroxyl functionality have the same geometry. The chemical shift in $CDCl_3$ solution for the Me-18 carbon in **2**, **3**, and **6** (17.0, 16.5, 18.8 ppm, respectively) appeared upfield relative to that for **1** (22.6 ppm), which bears no hydroxyl at C-2.[5] These data required that the relative configuration at C-2, as in the case of **2** and **3**, be *S** rather than *R** (i.e., 2-OH cis to Me-18). Due to the rigid cagelike nature of the tetracyclic carbon framework of elisapterosin (**6**), the aforementioned correlations were sufficient to establish the identity of the stereocenter at C-1 as *S**, which allowed elimination of numerous inconsistent possibilities. Thus, the overall relative stereochemistry for **6** was assigned as 1*S**, 2*S**, 3*S**, 6*R**, 7*S**, 9*S**, 10*S**, 15*S**, and 16*S**. The final confirmation of the determinations of the stereochemistry within **6** came from the single-crystal X-ray analysis of elisapterosin B (**7**), since repeated attempts to obtain suitable crystals from **6** were not successful. Elisapterosin B (**7**) is structurally very close to compound **6** and shows unusual NOE correlations very similar to those of **6** (vide infra).

Biosynthetically, elisapterosin A (**6**) could be envisioned as a precursor for elisabanolide (**4**) via enzyme-mediated oxidative cleavage of the 1,3-ketodiol functionality. Presumably, the ensuing carbonyl-forming cleavages should take place in vivo with concomitant elimination of C≡O (or H_2C═O) in a type of bisoxidative decarbonylation reaction, to give the *nor*-diterpene **4** (and **5**). On the other hand, compound **6** could undergo the reaction in vitro so readily due to the much higher thermodynamic stability of the expected products. Indeed, when a solution of elisapterosin A was studied by GC−MS analysis, complete decomposition took place, giving rise to at least five products. The identification of the two major components possessing the highest molecular ion peaks (each *m*/*z* 318) was established by comparison of the retention times and mass spectra recorded with those of authentic samples of elisabanolide (**4**) (R_t = 19.9 min) and 3-*epi*-elisabanolide (**5**) (R_t = 20.2 min) (see the Experimental Section). The identity of these products (obtained in a ratio of 1:2, respectively) was subsequently confirmed by peak enhancement experiments. Thus, thermally induced decomposition of **6** by loss of (presumably) formaldehyde leads primarily to *nor*-diterpenes **4** and **5** (Scheme 1). As far as we have been able to ascertain, this is the first example of such an oxidative rearrangement in natural products.[12]

Scheme 1[a]



[a] Reaction conditions: (a) 250 °C during GLC−MS analysis; (b) $Pb(OAc)_4$, benzene, pyridine, 80 °C, 22.5 h.

Once the novel skeleton of **6** was elucidated, the structure of its plausible natural precursor **7** was easy to assign. Elisapterosin B (**7**) is a white crystalline solid, $[\alpha]^{25}_D$ −3.0° (*c* 4.4, $CHCl_3$). Its molecular formula was determined to be $C_{20}H_{26}O_3$ by HREI-MS (*m*/*z* 314.1869) and differs from that of **6** by the loss of 34 Da. The missing units were deduced to be those of two hydroxyl groups. The precise elemental composition of **7** was corroborated subsequently by HRFAB-MS *m*/*z* 337.1779 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_3Na$, 337.1779). The IR data for **7** indicated the presence of hydroxyl (3409 cm^{-1}) and olefin (1612 cm^{-1}) functionalities. The carbon resonances at *δ* 203.8 and 193.0 coupled with strong IR absorptions at 1756 and 1661 cm^{-1} indicated two ketone functions, one of which was assigned to an α,*β*-unsaturated moiety on the basis of the UV band centered at 212 nm. The apparent chemical inertness of the ketone functionalities became evident when no satisfactory crystalline derivative could be prepared upon warming a mixture of **7** and *p*-bromophenylhydrazine to 45−50 °C for 2 days. As in the case of **1** and **2**, the presence of a vinyl hydroxyl group was suggested by the observation in the ^{1}H NMR spectrum of a sharp one-proton singlet at *δ* 6.10 as well as a ^{13}C NMR resonance at *δ* 146.1 (s). This assignment was further confirmed by the methylation of **7** at 25 °C with diazomethane to give the vinyl methyl ether derivative **8**. Thus, the signal at *δ* 6.10 in **7** was replaced by a sharp three-proton singlet at *δ* 3.71 in **8** ascribable to a vinyl methoxyl group. The remainder of the ^{1}H NMR spectrum could be matched with that of **7**. The ^{13}C NMR of **7** in $CDCl_3$ solution (Table 3) contained twenty signals including seven quaternary carbons and four methyl, five methine, and three methylene groups, one of which was a vinylic carbon (*δ* 114.7, C-12). The assignment of telltale elisapterane system resonances including those of the alkenyl side chain in compound **7** was entirely supported by 2D NMR experiments and confirmed by comparison with data for **6**. In the same way, the relative stereochemistry about the rings in elisapterosin B (**7**) was determined to be the same as that found in **6** by NOESY and monodimensional NOE experiments (Table 3). The

(11) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; International Series in Organic Chemistry; Pergamon Press: Oxford, U.K., 1969; Vol. 10, Chapter 4-2, pp 280−304.

(12) We also found that treatment of elisapterosin A (**6**) (3.7 mg, 0.011 mmol) with lead tetraacetate (9 mg, 0.02 mmol) in dry benzene (4 mL) and pyridine (2 drops) afforded the desired C_{19} diketones **4** and **5** in a 1:1 ratio after refluxing for 22.5 h at 80 °C as determined by TLC and GC−MS analyses.

Table 3. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Elisapterosin B (7) in $CDCl_3$[a]

position	δ_H, mult, intgr, (*J*, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		61.5 (s)			H6, H8*β*, H9, H10
2		141.5 (s)			H9, H18, 17-OH
3	3.20, m, 1H	28.4 (d)	H4α*β*, H18	H4*β*, H9, H18	H4α, H5α, H18
4α	1.50, m, 1H	24.2 (t)	H3, H4*β*, H5α*β*		H5*β*, H18
β	1.68, m, 1H		H3, H4α, H5α*β*	H3	
5α	1.53, m, 1H	19.2 (t)	H4α*β*, H5*β*, H6		H4*β*
β	1.77, m, 1H		H4α*β*, H5α, H6		
6	2.28, m, 1H	40.1 (d)	H5α*β*, H7	H19	H5α, H8*β*, H19
7	1.98, m, 1H	41.5 (d)	H6, H8α*β*, H19	H19	H5α*β*, H8α, H19
8α	0.84, q, 1H (11.9)	42.9 (t)	H7, H8*β*, H9	H10	H6, H10, H19
β	2.14, dt, 1H (5.7, 11.9)		H7, H8α, H9	H9	
9	2.42, dt, 1H (5.7, 11.9)	54.9 (d)	H8α*β*, H10	H3, H8*β*, H12*β*, H13	H6, H8α, H10
10	2.28, br d, 1H (5.7)	55.7 (d)	H9	H8α, H12*β*, H13, H20	H8α, H9, H12α*β*, H13, H20
11		142.5 (s)			H9, H10, H12*β*, H13
12α	4.86, br s, 1H	114.7 (t)	H12*β*, H13	H12*β*, H13	H10, H13
β	4.67, br s, 1H		H12α, H13	H9, H10, H12α	
13	1.63, br s, 3H	22.7 (q)	H12α*β*	H9, H10, H12α	H10, H12α*β*
14		203.8 (s)			H6, H9, H10, H20
15		70.1 (s)			H10, H20
16		193.0 (s)			H10, H20, 17-OH
17		146.1 (s)			17-OH
18	1.14, d, 3H (7.1)	17.6 (q)	H3	H3	H3, H4α*β*
19	1.03, d, 3H (6.4)	18.1 (q)	H7	H6, H7	H8α
20	1.41, s, 3H	13.3 (q)		H10	H10
17-OH	6.10, s, 1H				

[a] Chemical shift values are in parts per million relative to TMS. Spectra were recorded at room temperature. [b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences. [c] Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH} = 6$ and 8 Hz.

remaining NMR data of **7**, which greatly differed from those of **6**, suggested the presence of an isopropenyl and that of an α-keto enol functionality. In fact, the HMBC spectrum revealed long-range correlations between the C-17 hydroxyl at *δ* 6.10 and C-2 (*δ* 141.5), C-16 (*δ* 193.0), and C-17 (*δ* 146.1), as well as cross-peaks of C-2 with H-9 and Me-18. The Me-18 protons in turn showed COSY correlations with the methine proton at *δ* 3.20 (H-3). These combined correlations allowed the α-keto enol moiety to be inserted in a manner consistent with the molecular arrangement depicted in **7**. Additional HMBC correlations supporting this connection were those from Me-20 to C-14 (*δ* 203.8), C-16 (*δ* 193.0), and C-10 (*δ* 55.7). Finally, the long-range correlations of H_2-12 with C-10 and those between H-10 (*δ* 2.28) and C-12 (*δ* 114.7) allowed the isopropenyl side chain to be linked to C-10 and completed the unambiguous structural characterization of elisapterosin B. An X-ray diffraction experiment performed on **7** furnished a 3D ORTEP diagram (Figure 4) which fully supported the structure assigned on the basis of 2D NMR methods.

The results obtained from our previous investigations of *P. elisabethae* have demonstrated thus far that amphilectane metabolites co-occur with the serrulatane diterpenes as well as the *nor*-sandresane- and *norseco*-elisabethane-type compounds.[5−8] This would be consistent with a biosynthetic pathway wherein a serrulatane-based intermediate serves as a precursor to these rearranged terpenoid skeletons. Moreover, the isolation in the present work of all three skeletal classes, elisabethanes, elisapteranes, and elisabanes, from the same specimen of *P. elisabethae* provides circumstantial support that these ring systems might indeed be synthesized in vivo by subsequent cyclizations of the serrulatane (or amphilectane) skeleton (Scheme 2). Our results suggest that the biosynthesis of the elisapteranes most likely involve prior cyclization of a serrulatane-type diterpene followed by further cyclization of an intermediate elisabethin to an elisapterosin. Subsequent loss of a carbon atom (i.e., C_{17}) would account for the formation of the elisabane skeleton.

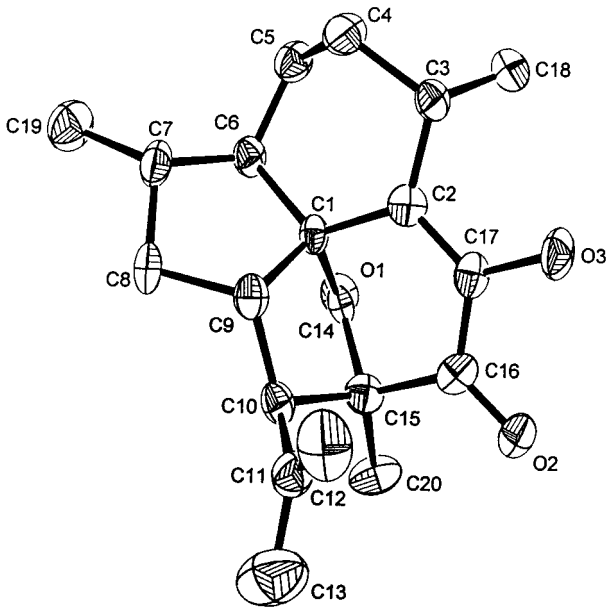
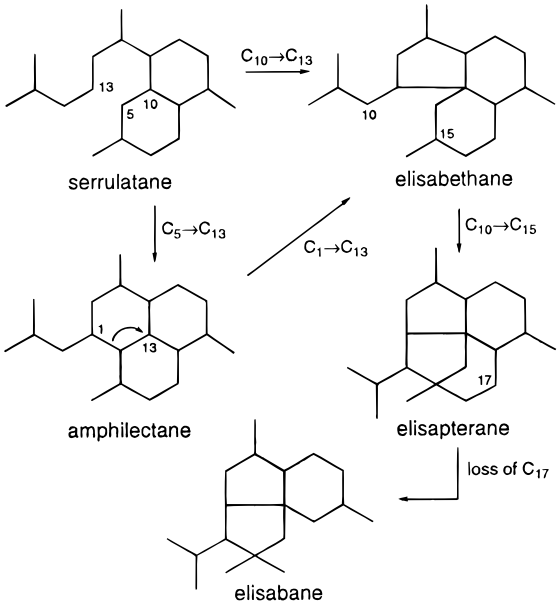


Figure 4. Computer-generated perspective drawing of the final X-ray crystallographic model of elisapterosin B (**7**). The thermal ellipsoids are drawn at the 40% probability level. No information could be interpreted to assign the absolute configurations at the asymmetric centers. The hydrogen atoms have been omitted for clarity.

Elisapterosin B (**7**) was submitted to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the Southern Research Institute for in vitro biological studies with *M. tuberculosis*. Compound **7** was found to effect strong inhibitory activity (79%) against *M. tuberculosis* H37Rv at a concentration of 12.5 *μ*g/mL. On the other hand, elisabethin D (**2**) proved inactive in

Scheme 2



the National Cancer Institute's (NCI's) test for agents active against the human inmunodeficiency virus (HIV). Follow-up biological screening of elisabethin D (**2**) and elisapterosin B (**7**) in the NCI's three-cell-line tumor panel indicated no significant in vitro CNS (SF-268), lung (NCI-H460), or breast (MCF7) cancer cell cytotoxicity. Additional experiments to assess the biological properties of compounds **1**−**7** are currently underway.

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 300 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. Normal-phase HPLC separations of natural products were performed on a Partisil 10 M9/50 silica gel (10 mm) column (9.5 × 500 mm) with 2% 2-propanol in hexane (flow rate, 2.0 mL/min; UV detector set at 220 nm). All solvents used either were spectral grade or were distilled from glass prior to use. A Hewlett-Packard 5890A gas chromatograph/5971 mass-selective detector was used for the GC−MS analyses. Diazomethane was prepared in-house according to literature procedures.[13] Diazald, acetic anhydride, pyridine, sodium acetate, *p*-bromophenylhydrazine hydrochloride, and lead tetraacetate were purchased from Aldrich Chemical Co. and were used without further purification. The percentage yield of each compound is based on the weight of the dry gorgonian MeOH−$CHCl_3$ extract.

Extraction and Isolation. The extraction scheme followed has been previously described.[5] 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 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. Having produced almost identical results during TLC and NMR analyses, subfractions 3.11 and 3.12 were combined (total weight, 4.30 g) and purified by column chromatography on silica gel (150 g) using a step gradient of 10−20% acetone in hexane as eluent. A total of 12 subfractions (A−L) were obtained. Elisapterosin B (**7**) (59.1 mg; 7.41×10^{-2}% yield) was obtained pure after subfractions D (167.1 mg) and E (268.6 mg) were chromatographed over silica gel (7.0 and 10.0 g, respectively) using step gradients of 5−10% EtOAc in hexane. Subfraction F (1.96 g) was chromatographed successively over silica gel (70 g) with 5% 2-propanol in hexane, then over ODS silica gel (20.0 g) with 5% H_2O in MeOH, and over silica gel (35 g) with 2% 2-propanol in hexane. Final purification was achieved by HPLC [Partisil 10 M9/50 silica gel eluting with 2% 2-propanol in hexane], which yielded pure elisabethin D acetate (**3**) (26 mg; 3.26×10^{-2}% yield). Subfraction 3.14 (173.6 mg), which was purified by column chromatography [silica gel (8.0 g) with 1% 2-propanol in $CHCl_3$], afforded elisabethin D (**2**) (27.1 mg; 3.40×10^{-2}% yield). Subfraction 3.16 (611.5 mg) was chromatographed successively over silica gel (25.0 g) with 15% EtOAc in hexane, then over ODS silica gel (6.0 g) with 15% H_2O in MeOH, and over silica gel (3.0 g) using $CHCl_3$ as eluent to yield pure 3-*epi*-elisabanolide (**5**) (5.8 mg; 7.27×10^{-3}% yield) and elisapterosin A (**6**) (20.3 mg; 2.54×10^{-2}% yield).

Elisabethin D (2): crystalline solid; $[\alpha]^{25}_D$ +6.4° (*c* 1.2, $CHCl_3$); UV (MeOH) λ_{max} 208 nm (ϵ 6100), 280 nm (ϵ 1300); IR (film) 3513, 3260, 2991, 1703, 1645, 1622, 1446, 1383, 1189, 1057, 984 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m*/*z* [M$^+$] calcd for $C_{20}H_{28}O_4$ 332.1987, found 332.1980 (27), 314.1886 (8, $C_{20}H_{26}O_3$), 304.2037 (13, $C_{19}H_{28}O_3$), 302.1885 (12, $C_{19}H_{26}O_3$), 233.1541 (41, $C_{15}H_{21}O_2$), 215.1433 (55, $C_{15}H_{19}O$), 193.0860 (67, $C_{11}H_{13}O_3$), 183.0655 (95, $C_9H_{11}O_4$), 177.0915 (75, $C_{11}H_{13}O_2$), 165.1277 (57, $C_{11}H_{17}O$), 109.1015 (87, C_8H_{13}).

Single-Crystal X-ray Diffraction Analysis of Elisabethin D. Crystallization of elisabethin D (**2**) by slow evaporation from a mixture of 1:1 hexanes−EtOAc yielded colorless block crystals of excellent quality. A specimen of 0.34 × 0.31 × 0.27 mm mounted on a glass fiber was selected for the analysis. X-ray diffraction data were collected on a Siemens SMART CCD system at 23 ± 1 °C to a maximum 2*q* value of 54.0°, using Mo *K*α radiation (λ = 0.710 69 Å). Preliminary X-ray photographs showed a primitive trigonal hexagonal cell with dimensions a = 10.6812(6) Å and c = 14.324(1) Å with V = 1415.2 (1) Å^3. The systematic extinctions, crystal density (d_{calcd} = 1.170 g/cm^3), and optical activity indicated space group $P3_1$ in the asymmetric unit (Z = 3) of composition $C_{20}H_{28}O_4$ with a formula weight of 332.44. Of the 7429 reflections measured, 1929 were unique (R_{int} = 0.022); equivalent reflections were merged. The crystallographic residual was R = 4.5% (R_w = 4.5%) 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. Hydrogen atoms were included but not refined. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corp.[14] Neutral atom scattering factors were taken from Cromer and Waber.[15]

Elisabethin D acetate (3): colorless oil; $[\alpha]^{25}_D$ +26.3° (*c* 2.8, $CHCl_3$); UV (CH_3OH) λ_{max} 208 nm (ϵ 7000), 252 nm (ϵ 4000); IR (film) 3498, 1771, 1711, 1683, 1654, 1456, 1373, 1195, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.27 (br d, 1H, J = 11.0 Hz, H-10), 2.82 (dt, 1H, J = 5.8, 11.0 Hz, H-9), 2.68 (br s, 1H, −O*H*), 2.53 (m, 1H, H-6), 2.46 (m, 1H, H-3), 2.27 (s, 3H, OCOC*H*$_3$), 1.88 (s, 3H, H-20), 1.72 (m, 1H, H-7), 1.53 (s, 3H, H-13), 1.45 (s, 3H, H-12), 1.15 (d, 3H, J = 6.5 Hz, H-19), 0.99 (d, 3H, J = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.4 (s, C-14), 190.5 (s, C-17), 168.4 (s, C-21), 151.6 (s, C-16), 134.9 (s, C-15), 133.6 (s, C-11), 125.2 (d, C-10), 80.5 (s, C-2), 70.2 (s, C-1), 44.9 (d, C-9), 44.7 (d, C-6), 40.1 (t, C-8), 38.0 (d, C-7), 27.6 (d, C-3), 25.9 (q, C-13), 25.2 (t, C-5), 24.9 (t, C-4), 20.2 (q, C-22), 18.9 (q, C-19), 17.6 (q, C-12), 16.5 (q, C-18), 9.5 (q, C-20);

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

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

(15) 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.

HREI-MS *m/z* [M$^+$] calcd for $C_{22}H_{30}O_5$ 374.2093, found 374.2061 (5), 314.1919 (23, $C_{20}H_{26}O_3$), 286.1971 (22, $C_{19}H_{26}O_2$), 223.0964 (87, $C_{12}H_{15}O_4$), 183.0685 (95, $C_9H_{11}O_4$), 177.0954 (73, $C_{11}H_{13}O_2$), 109.0991 (100, C_8H_{13}).

Reaction of Elisabethin D (2) with Acetic Anhydride. To 1.2 mg (0.0036 mmol) of elisabethin D was added Ac_2O (0.4 mL) in dry pyridine (1.0 mL). The resulting solution was stirred at 25 °C for 15 min and concentrated in vacuo, and the residue obtained was stored overnight under high vacuum. Purification by silica gel column chromatography followed to give a colorless oil identical in all respects to natural product **3**.

3-*epi*-Elisabanolide (5): crystalline solid; $[\alpha]^{25}_D$ −4.0° (*c* 1.5, $CHCl_3$); IR (film) 1769, 1730, 1691, 1454, 1375, 1260, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m/z* [M$^+$] calcd for $C_{19}H_{26}O_4$ 318.1831, found 318.1836 (42), 303.1645 (15, $C_{18}H_{23}O_4$), 290.1939 (42, $C_{18}H_{26}O_3$), 246.2040 (31, $C_{17}H_{26}O$), 231.1791 (30, $C_{16}H_{23}O$), 217.1608 (34, $C_{15}H_{21}O$), 203.1468 (36, $C_{14}H_{19}O$), 191.1426 (100, $C_{13}H_{19}O$), 189.1275 (56, $C_{13}H_{17}O$), 164.1207 (77, $C_{11}H_{16}O$), 135.1178 (56, $C_{10}H_{15}$).

Single-Crystal X-ray Diffraction Analysis of 3-*epi*-Elisabanolide. Crystallization of 3-*epi*-elisabanolide (**5**) by slow evaporation from a mixture of hexane−$CHCl_3$ yielded colorless crystals of excellent quality. A specimen of 0.45 × 0.35 × 0.15 mm selected for the analysis was mounted on a glass fiber. X-ray diffraction data were collected on a Siemens SMART CCD system at 173 ± 2 K to a maximum 2*q* of 27.1°, using Mo *K*α radiation (λ = 0.710 73 Å). Preliminary X-ray photographs showed orthorhombic symmetry, and accurate lattice constants of a = 7.5692(7) Å, b = 12.5375(11) Å, and c = 18.2151(16) Å. The systematic extinctions, crystal density (d_{calcd} = 1.223 g/cm^3), and optical activity indicated space group $P2_12_1$ in the asymmetric unit (Z = 4) of composition $C_{19}H_{26}O_4$ with a formula weight of 318.40. Of the 10739 reflections measured, 3803 were unique (R_{int} = 0.0371); equivalent reflections were merged. The crystallographic residual was R = 4.2% (R_w = 10.6%) 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. Hydrogen atoms were included but not refined. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corp.[14] Neutral atom scattering factors were taken from the *International Tables for X-ray Crystallography*.[15]

Elisapterosin A (6): crystalline solid; $[\alpha]^{25}_D$ +140.7° (*c* 1.4, $CHCl_3$); IR (film) 3467, 1746, 1722, 1458, 1382, 1097 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFAB-MS *m/z* [M + H]$^+$ calcd for $C_{20}H_{29}O_5$ 349.2015, found 349.1998; HREI-MS *m/z* [M$^+$] calcd for $C_{20}H_{28}O_5$ 348.1937, found 348.1944 (5), 320.1984 (3, $C_{19}H_{28}O_4$), 302.1925 (65, $C_{19}H_{26}O_3$), 258.2031 (19, $C_{18}H_{26}O$), 244.1529 (31, $C_{16}H_{20}O_2$), 219.1804 (95, $C_{15}H_{23}O$), 216.1529 (36, $C_{15}H_{20}O$), 193.0884 (100, $C_{11}H_{13}O_3$), 165.1314 (88, $C_{11}H_{17}O$), 137.0635 (66, $C_8H_9O_2$), 127.0790 (30, $C_7H_{11}O_2$), 109.1019 (42, C_8H_{13}).

Thermally Induced Decomposition of Elisapterosin A (6) during GC−MS Analysis. Analysis of a solution of elisapterosin A in $CDCl_3$ by TLC [silica gel UV254 precoated plates (0.25 mm) in 30% EtOAc in hexane] and ^{1}H NMR spectroscopy revealed that the starting material **6** was free of any major contaminant. After evaporation of the solution with N_2 followed by storage under high vacuum, a slightly yellow oily residue (1−2 mg) was obtained, which was subsequently dissolved in CH_2Cl_2 (500 μL) and analyzed directly by GC−MS using a Supelco Capillary SPB-5 column (30 m × 0.32 mm; program rate, 70−250 °C at 10 °C/min; injector temperature, 250 °C). While no trace of elisapterosin A (**6**) was detected, we tentatively identified two of the five major peaks as elisabanolide (**4**) and 3-*epi*-elisabanolide (**5**) on the basis of their GC retention times and mass spectra. Retention times for standard solutions of authentic elisabanolide (**4**) and 3-*epi*-elisabanolide (**5**) in CH_2Cl_2 after GC−MS analysis in the same manner (min): elisabanolide (19.9), 3-*epi*-elisabanolide (20.2). Retention times (min) of GC−MS major peaks in the solution of elisapterosin A (**6**): 15.6 (unknown; *m/z* 274), 16.9 (unknown; *m/z* 274), 19.1 (unknown; *m/z* 304), 19.8 (elisabanolide; *m/z* 318), 20.1 (3-*epi*-elisabanolide; *m/z* 318). The identity of the two products with the longest retention times (obtained in a ratio of 1:2, respectively) was subsequently confirmed by peak enhancement experiments.

Elisapterosin B (7): crystalline solid; $[\alpha]^{25}_D$ −3.0° (*c* 4.4, $CHCl_3$); UV (MeOH) λ_{max} 212 nm (ϵ 8500), 288 nm (ϵ 3400); IR (film) 3409, 1756, 1661, 1612, 1450, 1381, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 3); HRFAB-MS *m/z* [M + Na]$^+$ calcd for $C_{20}H_{26}O_3Na$ 337.1779, found 337.1779; HREI-MS *m/z* [M$^+$] calcd for $C_{20}H_{26}O_3$ 314.1882, found 314.1869 (4), 286.1950 (9, $C_{19}H_{26}O_2$), 271.1753 (11, $C_{18}H_{23}O_2$), 253.1633 (12, $C_{18}H_{21}O$), 206.0953 (100, $C_{12}H_{14}O_3$), 165.1289 (10, $C_{11}H_{17}O$).

Single-Crystal X-ray Diffraction Analysis of Elisapterosin B. Crystallization of elisapterosin B (**7**) by slow evaporation from methanol yielded colorless crystals of good quality. A specimen of 0.45 × 0.35 × 0.05 mm selected for the analysis was mounted on a glass fiber. X-ray diffraction data were collected on a Siemens SMART CCD system at 173 ± 2 K to a maximum 2*q* of 24.7°, using Mo *K*α radiation (λ = 0.710 70 Å). Preliminary X-ray photographs showed monoclinic symmetry, and accurate lattice constants of a = 6.884(3) Å, b = 12.897(5) Å, and c = 9.827(4) Å. The systematic extinctions, crystal density (d_{calcd} = 1.203 g/cm^3), and optical activity indicated space group $P2_1$ in the asymmetric unit (Z = 2) of composition $C_{20}H_{26}O_3$ with a formula weight of 314.41. Of the 4551 reflections measured, 2261 were unique (R_{int} = 0.0539); equivalent reflections were merged. The crystallographic residual was R = 7.8% (R_w = 19.4%) 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. Hydrogen atoms were included but not refined. All calculations were performed with the teXsan crystallographic software package of Molecular Structure Corp.[14] Neutral atom scattering factors were taken from the *International Tables for X-ray Crystallography*.[15]

Reaction of Elisapterosin B (7) with Diazomethane. After being treated with 10 mL of diazomethane solution in ether and stirred at room temperature for 2 days, compound **7** (9.8 mg, 0.03 mmol) was layered on top of a 10 cm × 10 cm plug of silica gel (1 g) in a sintered glass frit, and 30 mL of 5% ethyl acetate in hexane was poured through under aspirator vacuum. Concentration of the filtrate afforded pure product **8** (9.2 mg, 90%) as a viscous colorless oil. Data for **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.91 (br s, 1H, H-12α), 4.73 (br s, 1H, H-12*β*), 3.71 (s, 3H, OC*H*$_3$), 3.17 (m, 1H, H-3), 2.52 (dt, 1H, J = 6.0, 11.4 Hz, H-9), 2.29 (m, 1H, H-6), 2.27 (br d, 1H, J = 4.9 Hz, H-10), 2.14 (m, 1H, H-8*β*), 1.95 (m, 1H, H-7), 1.73 (br s, 3H, Me-13), 1.41 (s, 3H, Me-20), 1.10 (d, 3H, J = 7.1 Hz, Me-18), 1.04 (d, 3H, J = 6.4 Hz, Me-19), 0.83 (q, 1H, J = 12.1 Hz, H-8α); HREI-MS *m/z* [M]$^+$ calcd for $C_{21}H_{28}O_3$ 328.2038, found 328.2041 (100), 300 (43), 285 (20), 272 (73), 257 (31), 245 (19), 215 (12), 195 (24), 192 (25), 109 (11), 105 (11), 91 (16), 55 (34).

Attempted Reaction of Compound 7 with *p*-Bromophenylhydrazine·HCl. To a solution of *p*-bromophenylhydrazine hydrochloride (10.2 mg, 0.046 mmol) and sodium acetate (8 mg, 0.092 mmol) in water (0.5 mL) was added a solution of elisapterosin B (**7**) (11.2 mg, 0.036 mmol) in ethanol (1.5 mL). The resulting mixture was warmed to 45−50 °C under constant stirring for 2 days. No precipitate separated on cooling or upon careful dilution with water. TLC analysis of the colorless solution indicated no reaction.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra, interpretation of the HREI-MS data for elisapterosin A (**6**), and description of the X-ray crystal structure data, including large ORTEP drawings, tables of intramolecular distances, torsion angles, positional parameters, and intramolecular bond angles for elisabethin D (**2**), 3-*epi*-elisabanolide (**5**), and elisapterosin B (**7**). This material is available free of charge via the Internet at http://pubs.acs.org.

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