Pergamon

Tetrahedron Letters 41 (2000) 5177–5180

TETRAHEDRON LETTERS

Novel lactones from *Pseudopterogorgia elisabethae* (Bayer)†

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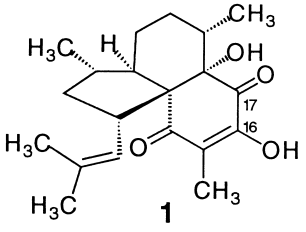
Received 30 March 2000; revised 28 April 2000; accepted 1 May 2000

Abstract

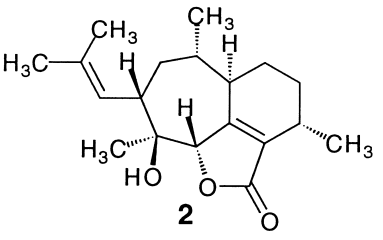
In addition to the previously reported terpenoids **1** and **2**, a chemical study of the hexane extracts of *Pseudopterogorgia elisabethae* led to the isolation of two novel skeletal lactones **3** and **4**. Their complete structures were established by interpretation of spectral data.

Keywords: coelenterates; marine metabolites; natural products; terpenes and terpenoids.

During our continuing studies on bioactive substances from West Indian gorgonians,[1] we isolated two known compounds, namely elisabethin D (**1**)[2] and sandresolide A (**2**),[3] in addition to two new tricyclic lactones named elisabetholide (**3**) and amphilectolide (**4**), from a Colombian specimen of *Pseudopterogorgia elisabethae* (Bayer) collected near San Andrés Island. Lactones **3** and **4** are structurally related to compounds **1** and **2**, respectively, but each possesses a novel carbon skeleton. This paper describes the isolation and structure elucidation of metabolites **3** and **4**.



1



2

After filtration, the 1:1 $CHCl_3$ and MeOH extract of dry *P. elisabethae* (1.0 kg) was subjected to gel filtration chromatography (Bio-Beads SX-3, toluene) followed by repetitive SiO_2 chromatography to afford the new lactones elisabetholide (**3**) (1.8 mg, 0.0006% dry wt) and amphilectolide

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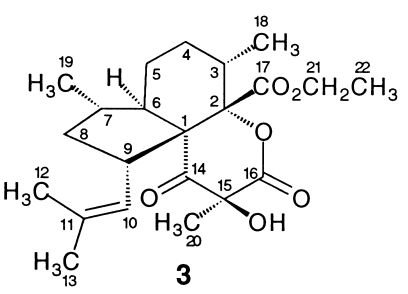
† Taken in part from the PhD Dissertation of C. Ramírez, University of Puerto Rico, 2000.

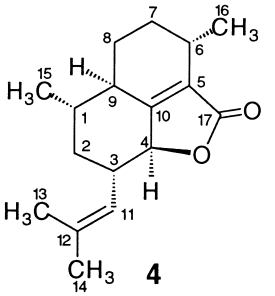
‡ Graduate student sponsored by the NIH-MBRS Program of the University of Puerto Rico.

§ Undergraduate student sponsored by the NIH-MBRS Program of the University of Puerto Rico.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00767-X

(**4**) (7.1 mg, 0.0025% dry wt). The structures of these metabolites were determined by interpretation of the 1D and 2D NMR (^{13}C, ^{1}H, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) and IR, UV, and HREI–MS spectra.





The IR spectrum of elisabetholide (**3**)[4] indicated the presence of hydroxyl (3500–3300 cm^{-1}) and carbonyl groups (1740 and 1720 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** showed the presence of a tertiary methyl [δ_H 1.63 (3H, s)], two secondary methyls [δ_H 0.96 (3H, d, $J=6.8$ Hz); 0.99 (3H, d, $J=5.9$ Hz)], a primary methyl [δ_H 1.33 (3H, t, $J=7.1$ Hz)], two vinyl methyls [δ_H 1.53, 1.55 (each 3H, br s)], a trisubstituted olefin [δ_H 4.67 (1H, br d, $J=10.1$ Hz)], three carbonyl groups [δ_C 209.8, 174.1, 171.5 (each s)], and three carbons bearing oxygen (two quaternary and one primary) [δ_C 93.2 (s), 84.4 (s) and 63.8 (t)] as shown in Table 1. Analysis of the ^{1}H–^{1}H COSY spectrum of **3** suggested the presence of partial structures (**A**) and (**B**) as shown in Fig. 1.

Table 1
^{1}H NMR, ^{13}C NMR, and HMBC spectral data for elisabetholide and amphilectolide[a]

Position	Elisabetholide (**3**)[b]			Amphilectolide (**4**)[c]		
	δ, mult (*J* in Hz)	^{13}C	HMBC	δ, mult (*J* in Hz)	^{13}C	HMBC
1		62.1, s	H8α, H9	1.20, m	39.5, d	H2αβ, H8α, H15
2α		93.2, s	H6, H9, H18	1.06, m	38.4, t	H11, H15
2β				1.56, m		
3	2.47, dq (2.5, 6.8)	38.1, d	H4β, H18	2.23, m	44.1, d	H1, H2αβ, H4, H11
4α	0.85, m	26.4, t	H3, H18	4.35, d (10.5)	83.6, d	H2αβ, H3, H11
4β	1.44, m					
5α	1.89, m	25.6, t	H3		128.1, s	H4, H7αβ, H16
5β	1.37, m					
6	2.01, m	50.1, d	H4αβ, H8α, H19	2.42, m	27.3, d	H8β, H16
7α	1.92, m	41.8, d	H6, H9, H19	1.86, m	31.2, t	H8β, H16
7β				1.13, m		
8α	1.16, m	41.5, t	H6, H9, H19	1.11, m	27.2, t	H9
8β	1.94, m			2.16, m		
9	3.12, dt (7.4, 10.1)	46.0, d	H8β	2.01, m	41.0, d	H1, H2αβ, H7αβ, H15
10	4.67, br d (10.1)	123.1, d	H8β, H11, H12		165.0, s	H3, H4, H8β, H9
11		134.1, s	H12, H13	5.07, br dd (1.2, 9.0)	125.2, d	H4, H13, H14
12	1.53, br s	18.0, q	H10, H13		134.5, s	H13, H14
13	1.55, br s	26.1, q	H10, H12	1.62, br d (0.9)	18.3, q	H11, H14
14		209.8, s	H20	1.72, br s	25.8, q	H11, H13
15		84.4, s	H20	1.04, d (6.3)	19.1, q	H1
16		171.5, s	H20	1.23, d (7.2)	17.8, q	H7αβ
17		174.1, s			172.9, s	
18	0.96, d (6.8)	15.8, q				
19	0.99, d (5.9)	18.2, q	H6, H7			
20	1.63, s	23.9, q				
21	4.34, dq (7.1, 17.6)	63.8, t	H22			
21'	4.10, dq (7.1, 17.6)					
22	1.33, t (7.1)	14.0, q	H21, H21'			

[a]Assignments were aided by ^{1}H-^{1}H COSY, spin splitting patterns, HMBC, HMQC and NOESY experiments, DEPT, 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. [b]Data recorded in $CDCl_3$ at 500 and 125 MHz. [c]Data recorded in $CDCl_3$ at 300 and 75 MHz.

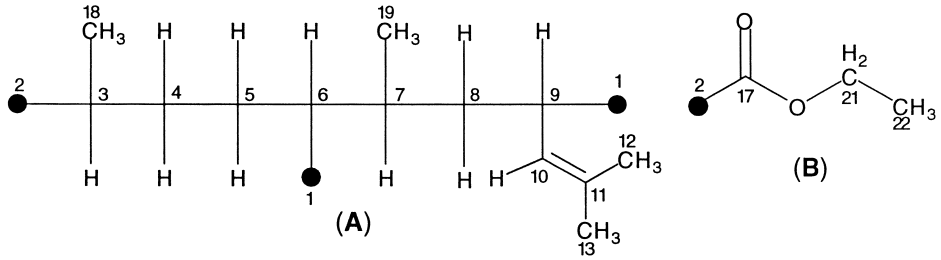


Figure 1. The partial structures of elisabetholide (**3**)

The new carbon skeleton was deduced by HMQC, DEPT, and NOESY along with the HMBC spectra as shown in Table 1. Segments of the ^{1}H and ^{13}C NMR spectra of **3** were quite similar to those of elisabethin D (**1**) except for the observation of a C-2 carbon signal [δ_C 93.2 (s)] in low field compared with a C-2 carbon signal [δ_C 78.3 (s)] of **1**, and a C-6 carbon signal [δ_C 50.1 (d)] in low field compared with a C-6 carbon signal [δ_C 43.7 (d)] of **1**.[2] Furthermore, the ^{1}H–^{1}H COSY, HMBC and NOESY spectra, which were reminiscent of those of **1**, indicated the presence of an ethoxycarbonyl group in **3**. To account for its chemical shift C-2 must bear both the ethoxycarbonyl and the oxygen of a lactone moiety. The stereocenters at C-2 and C-15 were deduced from observed NOE cross-peaks and *J* values for the ^{1}H NMR spectrum. A large coupling constant (10.1 Hz) between H-9 and H-10 indicated that these protons are nearly *trans* in a preferred conformation. A molecular modeling study revealed that in this conformation, when the ethoxycarbonyl group is β-oriented, it is possible to bring H-3 and Me-12 within observable NOE distance, in accord with the observed NOE. That the methylene protons of the ethoxycarbonyl group are diastereotopic implies restricted rotation thus indicating that there exists hydrogen bonding between this group and a nearby hydroxyl. On the basis of these combined observations we placed the hydroxyl at C-15 and the ethoxycarbonyl in the β-face of the molecule.[5] Thus, the relative structure of elisabetholide was determined as depicted in formula **3**. There is no doubt that compound **3** is not an artifact of the isolation procedure; a mixture of MeOH and $CHCl_3$ was used for extraction of the animal, and no ethanol was used in the isolation procedure.

Amphilectolide (**4**),[6] a rare trisnorditerpene, showed IR absorption bands for olefin (1678 cm^{-1}) and α,β-unsaturated lactone carbonyl (1745 cm^{-1}) functions. The UV (MeOH) spectrum showed absorption at λ_{max} 217 nm (log ε 3.48) indicative of conjugation. The molecular formula $C_{17}H_{24}O_2$, determined by the HREI–MS and ^{13}C NMR (Table 1), indicated six degrees of unsaturation. Resonances due to four olefinic carbons [δ_C 125.2 (d), 128.1 (s), 134.5 (s), 165.0 (s)] and a carbonyl carbon [δ 172.9 (s)] in the ^{13}C NMR spectrum accounted for three double-bond equivalents, indicating that **4** was tricyclic. The ^{1}H NMR spectrum (Table 1) indicated resonances corresponding to four methyls: two secondary [δ_H 1.04 (3H, d, $J=6.3$ Hz); 1.23 (3H, d, $J=7.2$ Hz)] and two vinyl methyl groups [δ_H 1.62 (3H, br d, $J=0.9$ Hz); 1.72 (3H, br s)], a trisubstituted olefin [δ_H 5.07 (1H, br dd, $J=1.2$, 9.0 Hz)], and an oxymethine group [δ_H 4.35 (1H, d, $J=10.5$ Hz)]. Analysis of the ^{1}H–^{1}H COSY spectrum suggested the presence in **4** of essentially the same partial structure (**A**) present in elisabetholide (**3**) (Fig. 1). In **4**, however, the signal ascribable to the allylic proton in substructure (**A**) (H-3) showed further coupling to an oxymethine signal at δ 4.35. Unlike those in elisabetholide, no additional spin systems were present in the ^{1}H–^{1}H COSY spectrum of **4**. The gross skeleton was completed by the HMQC and HMBC spectra (Table 1). The connectivity from C-4 to C-10 resulted from cross-peaks from H-4 to C-5 [δ 128.1, (s)] and

C-10 [δ 165.0, (s)]. The linkage from C-9 to C-10 and from C-5 to C-6 were inferred from long-range couplings from C-10 to H-8β [δ 2.16 (m)] and H-9 [δ 2.01 (m)] and from C-5 to H-7$\alpha\beta$ [δ 1.13, 1.86 (each m)] and Me-16 [δ 1.23 (d)], respectively. The carbonyl group was clearly in conjugation with a tetrasubstituted olefin on the basis of its UV absorption and the low chemical shift of C-10. Thus, compound **4** possessed two carbocyclic rings and one butenolide ring, as in the case of **2**.[3] The relative stereochemistry of **4** was deduced from NOESY correlations and *J* values. A large coupling constant ($J = 10.5$ Hz) indicated that H-3 and H-4 are *trans* to each other. NOEs from H-4 to H-2α and H-11, and from H-11 to H2α showed that these protons occur on the same face of the molecule (α). Likewise, NOEs from Me-15 to H-8α and H-9 indicated that these protons are all α-oriented opposite H-1, which in turn gave NOE correlations to both H-3 and H-8β. As in the case of **2**, the stereocenter at C-6, isolated from the rest of the molecule by two methylenes and the butenolide ring, was difficult to define by NOESY methods. The methyl group at C-6, however, was confidently assigned to the α-face of the molecule (i.e. *cis* to H-9) based on the NMR chemical shifts (in $CDCl_3$ solution) ascribed to Me-16 [δ_H 1.23 (d); δ_C 17.8 (q)], which were highly comparable to the known **2** [δ_H 1.25 (d); δ_C 19.3 (q)].[3] Further comparisons between these data and relevant NMR data in the pseudopterosin series (including those whose structures have been defined by X-ray methods) also suggested the C-6*S** relative configuration shown in structure **4**.[7] Thus, the overall relative stereochemistry for **4** was assigned as 1*S**,3*S**,4*S**,6*S**,9*R**.

Elisabetholide and amphilectolide, each possessing a new carbon skeleton from the gorgonian coral *P. elisabethae*, are the first examples in nature of such structural classes. Metabolite **3** might be biosynthesized from **1** through sequential oxidation at C-15, oxidative cleavage of the C_{16}–C_{17} bond, and intramolecular esterification. Amphilectolide can be considered as deriving from an amphilectane-based precursor by a series of oxidations and cleavages leading ultimately to the loss of carbons C-10, C-11, and C-20 (i.e. amphilectane numbering system). Compounds **2** and **4** induced 0 and 42%, respectively, growth inhibition for *Mycobacterium tuberculosis* $H_{37}Rv$ at a concentration of 6.25 μg/mL.

Acknowledgements

We thank the US Tuberculosis Facility (TAACF) for the antimycobacterial data. Janet Figueroa recorded the FT-IR spectra. Grants from NSF-MRCE and NIH-MBRS are gratefully acknowledged.

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4. For elisabetholide (**3**): a yellowish oil; $[\alpha]_D^{25}$ +21.9 (*c* 2.0, $CHCl_3$); IR (neat) 3500–3300, 2963, 2925, 2853, 1740, 1720, 1466, 1377, 1261, 1183, 1141, 972, 795 cm^{-1}; EIMS *m*/*z* $[M]^+$ 392 (7), 374 (1), 347 (17), 275 (24), 247 (31), 237 (100), 219 (24), 205 (34), 163 (36), 109 (65); HREIMS *m*/*z* $[M]^+$ 392.2184 (calcd for $C_{22}H_{32}O_6$, 392.2199).
5. The program Insight II (version 98.0) was employed for the molecular modeling studies.
6. For amphilectolide (**4**): a yellowish oil; $[\alpha]_D^{25}$ +24.7 (*c* 1.7, $CHCl_3$); IR (neat) 2955, 2929, 2856, 1745, 1678, 1457, 1378, 1265, 1126, 1029, 998 cm^{-1}; UV (MeOH) $\lambda_{max} = 217$ nm (log ε 3.48); EIMS *m*/*z* $[M]^+$ 260 (34), 205 (16), 161 (5), 109 (100), 91 (15), 77 (14), 67 (18), 55 (22); HREIMS *m*/*z* $[M]^+$ 260.1774 (calcd for $C_{17}H_{24}O_2$, 260.1776).
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