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Ileabethin: isolation and structure of a new class of perhydroacenaphthene diterpene from the Caribbean Sea Whip *Pseudopterogorgia elisabethae* (Bayer)

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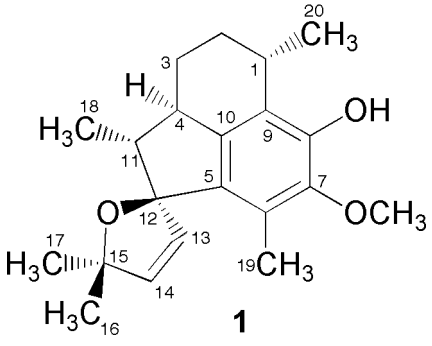
Abstract—A recent chemical investigation of the hexane extract of a Colombian specimen of *Pseudopterogorgia elisabethae* (Bayer) has led to the isolation of ileabethin (**1**). This novel diterpene possesses a previously undescribed carbon skeleton which appears to be biosynthetically related to the serrulatane (biflorane) skeleton. The structure of **1** was elucidated after interpretation of its combined spectroscopic data.

Gorgonian species belonging to the taxonomically complex *Pseudopterogorgia* genus constitute a bountiful source of pharmacologically active compounds.[1] For more than a decade *Pseudopterogorgia elisabethae* has been the subject of several chemical investigations which have led to the discovery of many structurally interesting and biologically active compounds (anti-inflammatory,[2] analgesic,[3] antituberculosis,[4] and antitumor[5]). The structural diversity found among the plethora of natural products isolated from *P. elisabethae* is indeed quite impressive.[6] Consequently, the natural products chemistry of this prolific marine animal continues to capture the attention of synthetic and biosynthetic chemists alike.

From the same organism, we have now isolated a new diterpene possessing an intriguing carbon framework. This compound, named ileabethin (**1**), contains an unprecedented perhydroacenaphthene carbon skeleton whose biogenetic origins could be traced back to the serrulatane (biflorane) family of diterpenes (Scheme 1).[7] Besides a new carbon skeleton, ileabethin (**1**) possesses several unusual structural features. On the one hand, there exists a fully substituted aromatic ring, whose substitution pattern resembles that of the pseudopterosin class of diterpene-glycosides, and on the other hand, the isobutenyl side chain found typically in many *P. elisabethae* metabolites appears in **1** masked as a spiro *gem*-dimethyl dihydrofuran moiety. This paper describes the isolation and structural characterization of ileabethin (**1**) which was based exclusively on the results of chemical and spectroscopic analysis.



1

After extraction with MeOH/$CHCl_3$ (1:1) of the sun-dried *P. elisabethae* (1.0 kg) collected in San Andrés Island, Colombia, a small portion (~27%) of the *n*-hexane extract was fractionated by successive size exclusion chromatography (Bio-Beads SX-3 in toluene) and SiO_2 chromatography leading to the isolation of pure **1** (2.2 mg, 0.0007% dry wt). The structure of ileabethin (**1**) was elucidated by interpretation of the data obtained from 1D and 2D NMR experiments and IR, UV and HREI MS spectral determinations.

Ileabethin (**1**) was obtained as a pale yellow oil; $[\alpha]_D^{25}$ +12.7 (*c* 1.1, $CHCl_3$). Eight degrees of unsaturation were deduced from its molecular formula $C_{21}H_{28}O_3$, established from HREI MS *m*/*z* $[M]^{+\bullet}$ 328.2053 (calcd

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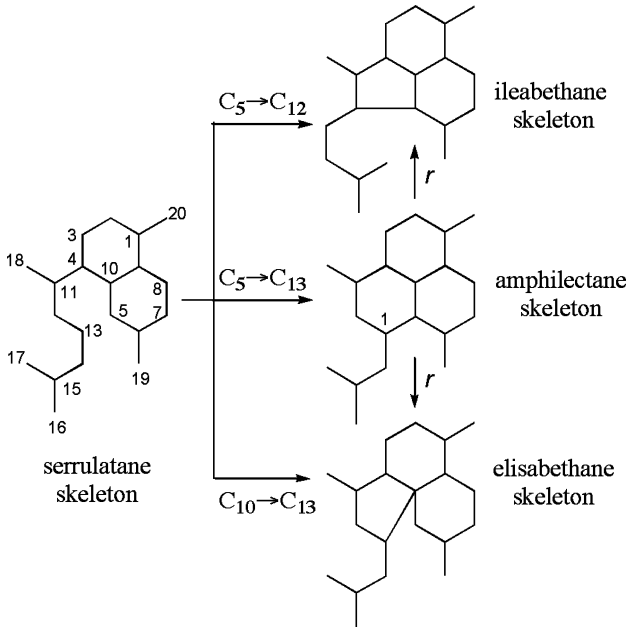

Scheme 1. Possible biogenetic interrelationships between the serrulatane, ileabethane, amphilectane, and elisabethane ring systems.

328.2038) and ^{13}C NMR measurements. The IR spectrum of **1** indicated the presence of a hydroxyl group (3500–3300 cm^{-1}) and the UV spectrum (MeOH) displayed a broad absorption between $\lambda_{max}=240$–280 nm suggesting the presence of a substituted benzene ring. This observation was supported by ^{13}C and DEPT NMR experiments ($CDCl_3$; 125 MHz) which, in addition to a 1,2-disubstituted olefin [δ_C 130.0 (CH) and 135.6 (CH)], exhibited six quaternary signals [δ_C 122.9 (C), 125.0 (C), 132.5 (C), 142.0 (C), 144.4 (C) and 147.2 (C)] ascribable to a fully substituted benzene ring. After substraction of all the unsaturations due to carbon–carbon double bonds, we concluded that ileabethin (**1**) must be tetracyclic.

The ^1H NMR spectrum ($CDCl_3$, 500 MHz) was consistent with the presumption that **1** was a diterpene (Table 1). There were five methyl signals, three of which were singlets displaced at δ_H 2.31, 1.42, and 1.40, designated as Me-19 (an aromatic methyl), Me-17 and Me-16, respectively. Two secondary methyl groups observed at δ_H 1.34 and 1.06 (each d, $J=6.8$ Hz) were ascribed to Me-20 and Me-18, respectively. A sixth methyl signal at 3.72 ppm (s, 3H) was readily assigned to an aromatic methoxyl and a sharp one-proton singlet at 5.78 ppm (D_2O exchangeable) hinted at a phenolic hydroxyl. Interestingly, while the latter functions accounted for two of the three oxygen atoms present in the molecular formula of **1**, there were two conspicuous oxygen-bearing carbons at δ_C 87.2 (C) and 100.4 (C) in the ^{13}C NMR spectrum. These quaternary carbon signals suggested to us that the remaining oxygen atom must be part of a spiro α,α′,δ,δ′-tetrasubstituted dihydrofuran ring. The strong downfield displacement observed for C-12 (δ_C 100.4) alluded to its ring size and bis allylic

Table 1. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), ^1H–^1H COSY, and HMBC spectral data of ileabethin (**1**)[a]

Position	δ_H mult, intgrt (J in Hz)	δ_C (mult)[b,c]	^1H–^1H COSY	HMBC[d]
1	2.92 ddq, 1H (6.8, 6.4, 4.5[e])	29.4 (CH)	H-2α, H-2β, Me-20	H-3α, Me-20
2α	2.17 m, 1H	33.5 (CH_2)	H-1, H-2β, H-3β	H-3α, Me-20
2β	1.37 m, 1H		H-1, H-2α, H-3β	
3α	2.06 m, 1H	28.3 (CH_2)	H-3β, H-4	
3β	0.96 m, 1H		H-2α, H-2β, H-3α, H-4	
4	2.60 ddd, 1H (11.4, 11.0, 4.4)	45.7 (CH)	H-3α, H-3β, H-11	H-3α, H-11, Me-18
5		125.0 (C)		Me-19
6		132.5 (C)		Me-19
7		144.4 (C)		Me-19, -OCH_3, 8-OH
8		147.2 (C)		8-OH
9		122.9 (C)		H-2α, Me-20, 8-OH
10		142.0 (C)		H-3α, H-11
11	1.62 m, 1H	52.0 (CH)	H-4, Me-18	Me-18
12		100.4 (C)		H-13, H-14, Me-18
13	5.66 d, 1H (5.9)	130.0 (CH)	H-14	H-14
14	5.88 d, 1H (5.9)	135.6 (CH)	H-13	H-13, Me-16, Me-17
15		87.2 (C)		H-13, H-14, Me-16, Me-17
16	1.40 s, 3H	28.5 (CH_3)		Me-17
17	1.42 s, 3H	28.7 (CH_3)		Me-16
18	1.06 d, 3H (6.8)	11.9 (CH_3)	H-11	
19	2.31 s, 3H	11.8 (CH_3)		
20	1.34 d, 3H (6.8)	21.0 (CH_3)	H-1	
-OCH_3	3.72 s, 3H	60.7 (CH_3)		
8-OH	5.78 s, 1H			

[a] NMR spectra were recorded in $CDCl_3$ at 25°C.
[b] ^1H and ^{13}C NMR chemical shift values are in ppm and are referenced to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals.
[c] ^{13}C NMR multiplicities were obtained by a DEPT experiment.
[d] Protons correlated to carbon resonances in ^{13}C column.
[e] Coupling constant obtained by an NMR simulation.

nature, thus reinforcing our contention that C-12 was indeed the spiro atom.

Other features of the ^{1}H NMR spectrum of **1** included two complex multiplets at δ 2.92 and 2.60 (each 1H) suggesting two benzylic hydrogens and a pair of mutually coupled olefinic protons appearing as doublets at δ_H 5.88 and 5.66 (each 1H, $J=5.9$ Hz). The small coupling constant suggested that such 1,2-disubstituted olefin was inside a five-membered ring. After assignments between all the direct C–H bonds were made by HMQC, the main connectivities allowing the entire structure elucidation of the carbocyclic framework of **1** were established by ^{1}H–^{1}H COSY and HMBC NMR experiments (Table 1).

Two separate ^{1}H–^{1}H spin systems were detected from the COSY NMR spectrum accounting for the two partial structures shown in Fig. 1. In addition, three partial structures (A–C) were deduced from extensive analyses of the 2D NMR data of **1** including COSY, HMQC, and HMBC spectra in $CDCl_3$ (Fig. 2). The HMBC experiment showed connectivity between the C-12 carbon [δ_C 100.4 (C)] and the protons of C-13 and C-14. The oxygen bearing quaternary carbon at position 15 (δ_C 87.2) showed strong HMBC correlations to H_3-16, H_3-17, and to the olefinic protons H-13 and H-14. Thus, the geminal methyls must be attached to

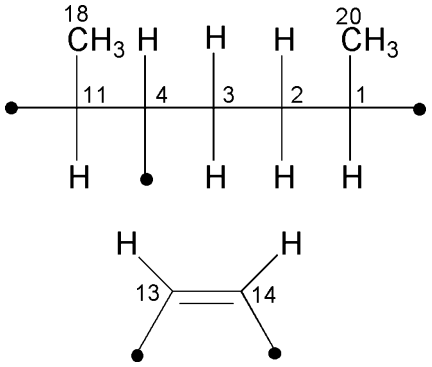

Figure 1. The proton spin systems of ileabethin (**1**).

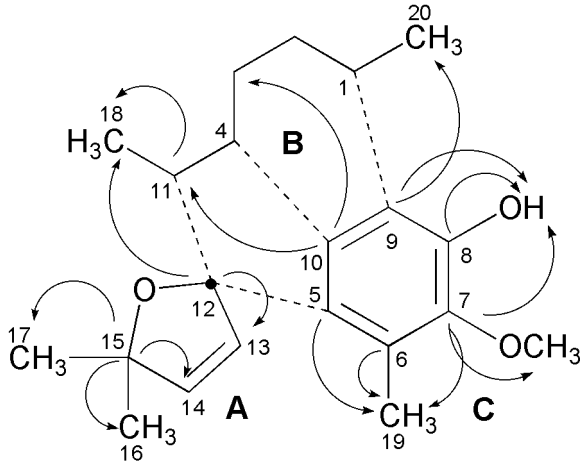

Figure 2. Selected HMBC correlations (^{13}C→^{1}H) for **1**.

C-15 thereby establishing the α,α′,δ,δ′-tetrasubstituted dihydrofuran substructure A.

Furthermore, substructures A and B were linked by a strong correlation between C-12 and the protons of C-18. Units B and C were connected by the observation of strong HMBC correlations of C-9 (δ_C 122.9) to H_3-20 and the hydroxyl proton which indicated that C-9 must lie between C-1 and C-8. From these data, we surmised that C-8 (δ_C 147.2) bears the OH group. Since C-7 (δ_C 144.4) also correlated strongly with 8-OH, H_3-19 and the -OCH_3 protons, this carbon has to be flanked by C-6 and C-8 and must bear the methoxyl group. Further HMBC correlations linking B and C were observed between the protons of C-19 with C-5 (δ_C 125.0) and C-6 (δ_C 132.5) and between C-10 (δ_C 142.0) and protons H-3α and H-11. Unfortunately, the attachment of the two rings of units A and C through C-5 and C-12 could not be established directly by HMBC since the expected three-bond proton–carbon connectivity between carbon resonance C-5 (δ_C 125.0) and H-13 (δ_H 5.66) could not be detected. Likewise, we did not detect any connectivity involving C-5 and protons H-4 (δ_H 2.60) or H-11 (δ_H 1.62). That notwithstanding, the link between units A and C through C-5 and C-12 (the only connecting points remaining) was clearly supported by the strong downfield displacement observed for C-12 in the ^{13}C NMR spectrum. This allowed the complete planar structure for **1** to be assigned.

The relative stereochemistry of **1** was established by a combination of NOESY data supported by distance calculations using the MacSpartan Pro molecular mechanics program, coupling constant analysis, and NMR spectral comparisons. Weak, but very diagnostic NOEs between H-2α with both H_3-20 and H-4 indicated that these protons were on the same face of the molecule and were assigned as the α protons. Likewise, H_3-18 showed an NOE response with H-3α, but not with H-3β, confirming the α-orientation for the C-18 methyl protons. Similarly, NOESY correlations between H-13 and H-14, and between H_3-19 and the -OCH_3 group, established the spatial proximities of these protons. Furthermore, the proton at C-4 occurred as a doublet of doublets of doublets exhibiting large couplings with H-3β and H-11 ($J=11.4$ and 11.0 Hz, respectively). These coupling constants indicated that these protons were *trans*-coupled. On the other hand, the small axial–equatorial coupling constant between H-3α and H-4 ($J=4.4$ Hz) indicated that these protons were *cis*-coupled. In agreement with the proposed relative stereochemistry at C-1, the larger axial–axial coupling constant between H-1 and H-2α ($J=6.4$ Hz) indicated these protons to be *trans*-coupled whereas the smaller axial–equatorial coupling constant between H-1 and H-2β ($J=4.5$ Hz) required the latter protons to be *cis*-coupled. Although the C-12 stereocenter was difficult to define by NOESY methods, the C-12*S** configuration shown in **1** was assigned confidently based on the following observations.[8] In the ^{1}H NMR spectrum the aromatic H_3-19 methyl appears unusually deshielded to 2.31 ppm which suggests that an oxygen

atom in very close steric proximity (β-oriented) is causing a pronounced downfield shift (generally 0.3–0.5 ppm).[9] Moreover, the fact that the aromatic C-19 methyl in **1** appears shielded to 11.8 ppm implies that the π system of the spiro dihydrofuran ring is α-oriented.[10] These results definitely supported the relative stereochemistry of **1** with the cyclohexane and cyclopentane rings having the half-chair and envelope conformation, respectively. Thus, the overall relative stereochemistry for ileabethin (**1**) was assigned as 1*S**, 4*R**, 11*R**, 12*S**.

Ileabethin (**1**) slowly decomposed upon prolonged storage in $CDCl_3$ at 25°C, which precluded our probing its biological properties. In view of the novel structure of **1**, we sought to obtain additional quantities from the remaining *n*-hexane extract in order to ascertain its biological properties. However, no discernable quantities of ileabethin could be isolated from this source, a fact that makes the recollection of a larger sample of *P. elisabethae* an utmost necessity.

Acknowledgements

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8. Arguably, the absence of NOEs between the vinylic H-13 proton and H-11 or H_3-19 implies that H-13 is not within spatial proximity to these protons, a condition that, in accord with a molecular modeling study, is more closely attainable when C-12 has the *S** configuration.
9. Typically, in the amphilectane-based pseudopterosin series (Scheme 1), the aromatic methyl group resonates between 2.00 and 1.90 ppm in $CDCl_3$. The close steric proximity of the dihydrofuran ring oxygen to the H_3-19 methyl in **1**, clearly revealed by space filling molecular models, leads to deshielding. For additional examples of the so-called intramolecular van der Waal's shift see Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance in Organic Chemistry*; Pergamon Press: Oxford, 1978; Vol. 10, pp. 70–72.
10. For instance, in pseudopterosins A–D, each having a β-oriented isobutenyl group at the C-1 position, the corresponding aromatic methyl groups resonate at 20.9 ppm in $CDCl_3$. On the other hand, in pseudopterosins K and L, where the isobutenyl group is α-oriented, the aromatic methyls resonate at 10.8 ppm. See references 2 and 3(b).