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Diterpenoids from Cultured *Erythropodium caribaeorum*

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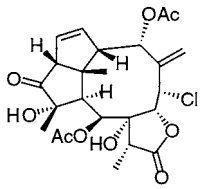
ABSTRACT



Aquariolide A

The known antimitotic agent eleutherobin and the briarane diterpenoids erythrolides A and B have been isolated from cultured specimens of *Erythropodium caribaeorum* in amounts comparable to those reported from wild-harvested reef animals. The novel diterpenoid aquariolide A, having an unprecedented highly rearranged carbon skeleton (named aquariane), has also been found. The aquariane skeleton can be formally derived from the briarane skeleton by sequential di-π-methane and vinyl-cyclopropane rearrangements.

Producing an adequate and sustainable supply of compounds represents one of the major challenges to developing invertebrate-derived marine natural product drug leads into clinically useful entities.[1] Harvesting invertebrate specimens from the wild is not a viable source of a drug substance because it is environmentally unsound and unpredictable. The obvious and preferred solution to this supply problem is total synthesis of the natural product or simplified analogues.[2] However, many marine natural products with promising biological activity are too complex to be readily synthesized on a commercial scale[3,4] and frequently the minimal pharmacophore is also a daunting synthetic challenge.[5] Aquaculture represents an attractive intermediate solution that utilizes the biosynthetic capabilities of the source organism to generate the compound of interest in a controlled and renewable fashion. Despite the appeal of aquaculture, the difficulties associated with growing marine invertebrates in culture has thus far limited this approach to a few examples.[1]

Eleutherobin (**1**) is a microtubule-stabilizing diterpenoid glycoside originally isolated from the rare Australian octocoral *Eleutherobia* sp.[6] Although two elegant total syntheses

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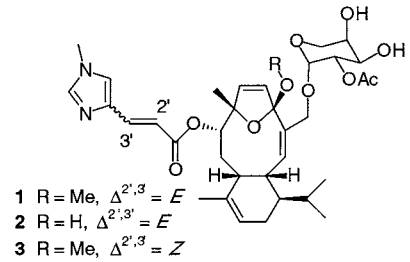
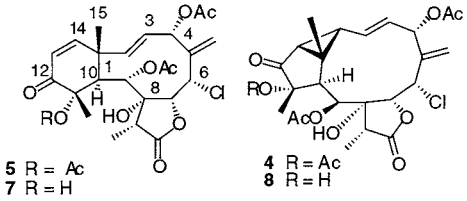
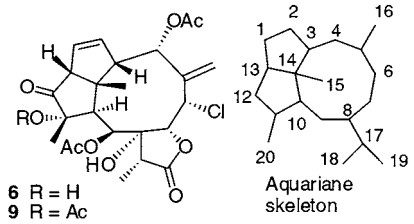
of eleutherobin (**1**) have been reported,[4] the anticancer potential of eleutherobin has never been fully evaluated because total synthesis and the original natural source both failed to provide sufficient material for effective in vivo testing.





Recently, it was discovered that *Erythropodium caribaeorum*, a relatively abundant Caribbean gorgonian, is a good source of eleuthesides,[7] and it has provided sufficient eleutherobin (**1**) for preliminary animal studies[8] and chemical transformations to new analogues.[9] *E. caribaeorum* grows well in culture and is a staple of the decorative seawater aquarium industry. We have examined methanol extracts of cultured *E. caribaeorum* to determine if these animals produce the diterpenoids found in wild animals. The extracts yielded eleutherobin (**1**), desmethyleleutherobin (**2**), (*Z*)-eleutherobin (**3**), erythrolide A (**4**),[10] and erythrolide B (**5**) in amounts comparable to those reported from Dominican reef animals,[7] along with aquariolide A (**6**), which has not been reported to date from field-harvested *E. caribaeorum*.[7,10,11] Aquariolide A (**6**) has an unprecedented highly rearranged diterpenoid carbon skeleton[12] that can be formally derived from the "briarane" skeleton found in erythrolide B (**5**) by sequential di-π-methane and vinyl-cyclopropane rearrangements (VCR).

Cultured *E. caribaeorum* was grown on artificial rocks in shallow running seawater tanks located in a greenhouse under ambient sunlight illumination. The animals used in the study were several generations removed from the wild stock. Freshly harvested animals (400 g wet wt) were shipped to Vancouver live in chilled seawater, cut into small pieces, and extracted multiple times with MeOH. Fractionation of the combined MeOH extracts via solvent partitioning, silica gel flash chromatography, and normal-phase HPLC (for the detailed isolation procedure, see Supporting Information) gave **1** (5.0 mg), **2** (1.2 mg), **3** (0.8 mg), **4** (290 mg), **5** (1.4 g), and **6** (3.0 mg). The known compounds **1**−**5** were identified by comparison of their spectroscopic data with literature values.[7,10]



Aquariolide A (**6**) was isolated as a colorless glass that gave [M^+] and [$M^+ + 2$] ions at *m*/*z* 496.1498 and 498.1487 (~3:1) in the HREIMS consistent with a molecular formula of $C_{24}H_{29}O_9Cl$, requiring 10 sites of unsaturation. One fragment of **6**, stretching in a continuous sequence from the acetoxy methine at C-4 (^{1}H: δ 5.71) along the linear carbon chain to the methine at C-10 (^{1}H: δ 2.70), including the α-methyl-γ-lactone fused at C-7/C-8, was shown to be identical to the corresponding C-4 to C-10 fragment in erythrolide B (**5**) by detailed analysis of the one- and two-dimensional NMR data obtained for **6** (Table 1 and Supporting Information). COSY correlations observed between H-4 (δ5.71) and an aliphatic methine resonance at δ 3.03, assigned to H-3, identified the first point of difference between **6** and **5**. Additional COSY correlations extended this spin system from H-3 through the vicinal cis olefinic protons H-2 (δ 5.82) and H-1 (δ 6.11) to its terminus at the aliphatic methine H-13 (δ 3.43). The H-3 resonance showed allylic coupling to H-1 and homoallylic coupling to H-13 in the COSY spectrum, consistent with the assigned spin system. With the ^{1}H assignments in hand, HMQC correlations permitted routine assignments of ^{13}C resonances to C-3 (δ 63.1), C-2 (δ 126.8), C-1 (δ 131.5), C-13 (δ 69.4), and C-10 (δ 43.5).

A methyl singlet resonance at δ 1.60 (Me-15) showed HMBC correlations to a quaternary carbon resonance at δ 49.6 (C-14) and to the C-3, C-13, and C-10 resonances, which required that C-3, C-13, and C-10 all be attached to the carbon (C-14) bearing the methyl group. This set of connectivities established the presence of a nine-membered ring in **6** that is fused to a cyclopentene ring at C-3 and C-14.

A second methyl singlet at δ 1.12 (Me-20) also showed HMBC correlations to the C-10 resonance (δ 43.5) and to nonprotonated carbons at δ 79.6 (C-11) and 209.0 (C-12), demonstrating that the oxygenated tertiary carbon (C-11)

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(12) We propose the name "aquariane" for this new carbon skeleton and a numbering scheme that preserves the C-4 to C-12 numbering of the briarane and erythrane skeletons.

Table 1. NMR Data for Aquariolide A (**6**) Recorded in $CDCl_3$ at 500 MHz (^1H) and 100 MHz (^{13}C)

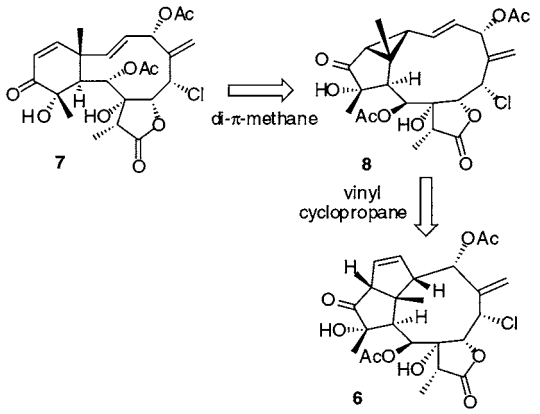
carbon no.	δ ^1H, multiplet, J in Hz	δ ^{13}C
1	6.11, m	131.5
2	5.82, ddd, 7.0, 2.7, 2.0	126.8
3	3.03, m	63.1
4	5.71, bd, 3.0	68.0
5		140.0
6	5.30, ddd, 4.7, 2.0, 1.8	62.2
7	5.48, d, 4.7	77.7
8		82.2
8-OH	2.87, s	
9	5.59, d, 4.0	67.7
10	2.70, d, 4.0	43.5
11		79.6
11-OH	2.05, bs	
12		209.0
13	3.43, m	69.4
14		49.6
15	1.60, s	28.7
16a	6.03, bd, 2.0	123.6
16b	6.00, bs	
17	2.51, q, 7.3	43.2
18	1.21, d, 7.3	7.0
19		174.8
20	1.12, s	21.7
4-Ac	2.10, s	21.1
		170.8
9-Ac	2.23, s	21.6
		169.7

bearing the methyl group (Me-20) was situated between C-10 and the ketone (C-12). The fragments identified above account for all of the atoms in aquariolide, except for a single hydrogen that was not attached to carbon, and they account for 9 of the 10 sites of unsaturation. Therefore, the ketone (C-12) had to be attached to C-13 to generate a five-membered ring representing the final site of unsaturation, and the oxygen functionality at C-11 had to be an alcohol to accommodate the final hydrogen atom. The cyclopentanone ring so formed mirrors the one present in erythrolide A (**4**).

The relative stereochemistry of **6** was deduced from the ROESY data. All the dipolar couplings observed for the C-4 to C-12 fragment were in complete agreement with the stereochemistry reported for the same region in erythrolides A (**4**) and B (**5**). In particular, the following cross-peaks were detected: H-4/H-6; H-6/H-7; Me-18/OH-8; H-7/*Me*COO-9; H-10/OH-8; H-9/Me-20; OH-11/Me-20. Transannular NOEs between OH-8 and H-16a and between H-10 and H-16b were also detected. Analysis of molecular models revealed that **6** can readily adopt a nine-membered ring conformation where the olefinic methylene protons H-16a and H-16b sit close to OH-8 and H-10, respectively. ROESY correlations observed between Me-15 (δ 1.60) and both H-3 (δ 3.03) and H-13 (δ 3.43) indicated a cis relationship for all of these protons. Furthermore, a ROESY correlation between H-3 (δ 3.03) and H-4 (δ 5.71) and their scalar coupling of only 3.0 Hz were only consistent with the placement of these protons on the same face of the nine-membered ring. Fixing the H-3/H-4 relative stereochemistry correlated the relative stereochemistry of the cyclopentene ring with the remaining part of the molecule. Assuming that the C-4 to C-12 fragment of aquariolide A (**6**) has the same absolute configuration as the corresponding fragments in erythrolides A (**4**) and B (**5**),[10] the absolute configuration of aquariolide A is as shown in **6**. Aquariolide A (**6**) showed modest in vitro cytotoxicity (IC_{50} 8 μg/mL) against MCF7 human breast cancer cells.

The new aquariane skeleton can be formally derived from a putative briarane precursor **7** (Scheme 1). A di-π-methane

Scheme 1. Proposed Biogenesis of Aquariolide A (**6**) from a Putative Briarane Precursor **7**



rearrangement of **7** gives an erythrane intermediate **8**, which can undergo a VCR to give the aquariane skeleton in **6**. Fenical et al. showed that erythrolide B (**5**) can be converted to erythrolide A (**4**) by irradiation with ambient sunlight.[10] Although most VCRs require high temperatures, there are reports of photochemical variants.[13] Side by side exposure of solutions of erythrolide B (**5**) and erythrolide A (**4**) in MeOH, acetone,[13] and toluene[13] to ambient sunlight resulted in the expected transformation of **5** to **4** in all solvents but produced no detectable change in **4** in any of the three solvents.[14] Therefore, it seems that the VCR required to convert an erythrane such as **8** into an aquariane (i.e., **6**) must be enzyme mediated. The absence of detectable amounts of the 11-acetoxy aquariolide A analogue **9** in the cultured *E. caribaeorum* extracts supports this conclusion. Erythrolide A (**4**) is a major component of the extract, and if its conversion to an aquariolide occurred via a nonenzymatic photochemical process, then **9** should have been a significant component of the extractable diterpenoids.

Our finding that cultured *E. caribaeorum* produces eleutherosides opens the way to large-scale aquaculture production of these interesting antimitotic agents should they prove to

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(14) This result is consistent with Fenical's report (ref 10) that irradiation of a benzene solution of erythrolide B (**5**) in a quartz cell with a Hg lamp gave the single product erythrolide A (**4**).

be viable anticancer drugs. The parallel discovery of aquariolide A (**6**) adds a new dimension to our understanding of the diterpenoid chemistry of *E. caribaeorum*. It suggests that the conversion of briaranes (i.e., **5**) to erythranes (i.e., **4**) is not simply a tolerated happenstance photochemical event but an integral part of the metabolic pathways of the gorgonian, since there must be enzymes that have evolved to transform erythranes (i.e., **8**) into aquarianes (i.e., **6**).

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Supporting Information Available: NMR spectra, tables of NMR assignments, physical constants, and isolation details for Aquariolide A (**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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