Briarane, Erythrane, and Aquariane Diterpenoids from the Caribbean Gorgonian *Erythropodium caribaeorum*

Orazio Taglialatela-Scafati,[a,b] Kyle S. Craig,[a] Delphine Rebérioux,[c] Michel Roberge,*[c] and Raymond J. Andersen*[a]

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Seven new diterpenoids belonging to the briarane [erythrolides R (**5**), S (**6**), T (**7**), and U (**8**)], erythrane [erythrolide V (**9**)], and aquariane [aquariolides B (**10**) and C (**11**)] skeletal classes have been isolated from the encrusting gorgonian *Erythropodium caribaeorum*. The structures of the new compounds were elucidated by spectroscopic analysis and chemical interconversions. A biogenetic proposal that links carbon skeletons found in *E. caribaeorum* diterpenoids is presented. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2003)

Introduction

Chemical investigations of marine coelenterates belonging to the subclass Octocorallia have unveiled an incredible array of novel diterpenoids, including many that have unprecedented carbon skeletons.[1] The encrusting gorgonian coral *Erythropodium caribaeorum* (Briareidae, Gorgonaceae) is the source of erythrolides A (**1**) and B (**2**),[2a] two chlorinated diterpenoids that are interrelated by a di-π-methane rearrangement. Since the initial report of **1** and **2**, eighteen additional erythrolide analogues (erythrolides C to Q, 3-acetylerythrolides E and I, and 16-acetylerythrolide H) have been isolated from *E. caribaeorum*.[2]

The recent discovery that *E. caribaeorum* produces significant amounts of the potent antimitotic agent eleutherobin (**3**),[3] a microtubule stabilizing agent that binds to the paclitaxel site on tubulin, has generated renewed interest in this relatively common gorgonian. Prior to its discovery in *E. caribaeorum*, the preclinical evaluation of eleutherobin was thwarted by the failure of both the original natural source (the rare alcyonacean *Eleutherobia* sp.)[4] and total synthesis[5] to provide enough material for definitive animal testing. While wild-harvest of *E. caribaeorum* is a good interim source of eleutherobin, environmental concerns would prohibit a wild-harvest source from supporting the long-term clinical use of the compound. Therefore, the possibility of effectively growing *E. caribaeorum* in culture would be of pivotal importance for the large-scale sustainable production of eleutherobin. Towards this goal, we have recently shown that cultured specimens of *E. caribaeorum*, several generations removed from the wild stock, continue to produce eleutherobin in roughly the same amounts as found in wild specimens.[6] During the analysis of aquarium grown *E. caribaeorum*, we also isolated aquariolide A (**4**), a highly rearranged diterpenoid having the unprecedented "aquariane" carbon skeleton.[6]

In order to obtain additional amounts of eleutherobin (**3**) for further pharmacological and semi-synthetic studies,[3c] and to assess the presence of aquariolide A (**4**) in wild animals, new specimens of *E. caribaeorum* were collected along the coasts of Dominica. A careful analysis of the diterpenoid fraction obtained from the organic extract of these specimens confirmed the presence of aquariolide A (**4**) in the wild animals and led to the isolation of many known (erythrolides A, B, E, H, J, M, P, Q; 16-acetylerythrolide H) and seven new diterpenoids, whose structures are presented in this paper. The new diterpenoids belong to the briarane [erythrolides R (**5**), S (**6**), T (**7**), and U (**8**)], erythrane [erythrolide V (**9**)], and aquariane [aquariolides B (**10**) and C (**11**)] skeletal classes.

[a] Departments of Chemistry and Earth and Ocean Sciences, University of British Columbia,
Vancouver, B.C. V6T 1Z1, Canada
Fax (internat.): +001-(0)11-6048226091
Email: randersn@interchange.ubc.ca
[b] Permanent address: Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II",
Via D. Montesano 49, 80131 Napoli, Italy
[c] Department of Biochemistry and Molecular Biology, University of British Columbia,
Vancouver, B.C. V6T 1Z3, Canada

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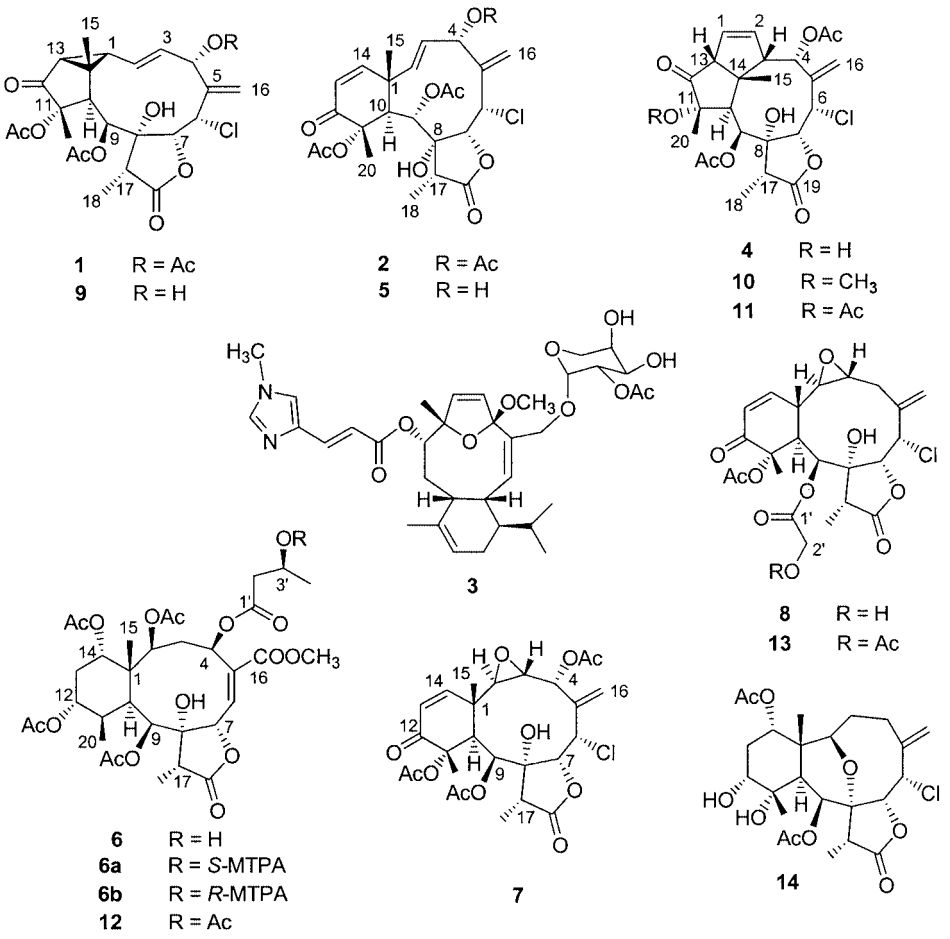


Results and Discussion

Freshly collected specimens of *E. caribaeorum* were frozen on site and transported to Vancouver at 0 °C. Thawed samples (4.8 kg wet wt.) were cut into small pieces and extracted multiple times with fresh MeOH. The combined MeOH extracts were concentrated in vacuo to give a brown-colored gum, which was partitioned between EtOAc and water. Further partitioning of the EtOAc-soluble materials between *n*-hexane and MeOH/H_2O, 9:1, yielded 32.5 g of MeOH/H_2O soluble compounds. Fractionation of the MeOH/H_2O soluble compounds by sequential application of normal-phase flash (gradient elution from *n*-hexane/EtOAc, 6:4, to EtOAc to EtOAc/MeOH, 1:1, in 5% increments) and normal-phase high-performance liquid chromatographies gave pure samples of erythrolides A (**1**),[2a] B (**2**),[2a] E,[2b] H,[2b] J (**12**),[2c] M,[2f] P (**14**),[2f] Q,[2f] 16-acetylerythrolide H,[2e] and aquariolide A (**4**).[6] These known diterpenoids were identified by comparison of their spectroscopic data with those reported in the literature. Seven new diterpenoids were also isolated and named erythrolide R (**5**, 23 mg), S (**6**, 3 mg), T (**7**, 1 mg), U (**8**, 1 mg), and V (**9**, 3 mg), and aquariolide B (**10** 4 mg) and C (**11**, 1 mg).

Erythrolide R (**5**) was isolated as a pale yellow oil that gave a $[M + H]^+$ ion at *m/z* = 497.1579 in the HRCIMS consistent with a molecular formula of $C_{24}H_{29}{}^{35}ClO_9$ (calcd. for $C_{24}H_{30}ClO_9$; 497.1578). Both the ^1H and ^{13}C NMR spectra of **5** (Tables 1 and 2, respectively), fully assigned through analysis of 2D NMR spectroscopic data (COSY, HMQC, and HMBC), closely resemble those reported for erythrolide B (**2**).[2a] In particular, the differences in the ^1H NMR spectrum of **5** are confined to the lack of an acetyl methyl signal, to the substantial upfield shift of 4-H (δ = 4.71 instead of δ = 5.68 ppm), and to a small shift of the signals attributed to the protons neighboring 4-H. These differences, in agreement with the molecular formula and ^{13}C NMR spectroscopic data, indicate that erythrolide R (**5**) is the 4-desacetyl analogue of erythrolide B. This conclusion was confirmed by treatment of erythrolide R (**5**) with Ac_2O in pyridine at room temp. which converted it into a compound showing spectroscopic data (NMR and $[\alpha]_D$) identical to those reported for erythrolide B (**2**).

Erythrolide S (**6**) was isolated as a pale yellow amorphous solid that gave a $[M + NH_4]^+$ ion at *m/z* = 716.3126 in the HRCIMS appropriate for a molecular formula of $C_{33}H_{46}O_{16}$ (calcd. for $C_{33}H_{50}NO_{16}$; 716.3129). Detailed analysis of 2D NMR spectroscopic data (COSY, HMQC, and HMBC) was used to completely assign the ^1H and ^{13}C NMR spectra of **6** (Tables 1 and 2, respectively). This identified the constitution of **6** as that of a new erythrolide bearing acetoxy groups at C-2, C-9, C-12, and C-14, respectively, a 3-hydroxybutanoyl moiety at C-4, and a $\Delta^{5,6}$ double bond. Compound **6** is very closely related to erythrolide J (**12**), reported by Dookran et al.,[2c] which differs from **6**

Table 1. ^{1}H NMR (500 MHz) data for erythrolides R−V (**5**−**9**)

Pos.	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]
1					2.37, dd, 8.5, 7.0
2	6.09, d, 15.2	4.86, dd, 9.0, 2.2	3.24, br. s	3.07[b]	6.37, dd, 17.8, 7.0
3a	5.46[b]	2.91[b]	3.69, br. d, 3.2	3.43, m	5.98, dd, 17.8, 2.5
3b		2.19[b]			
4a	4.71, d, 8.5	5.81, dd, 12.0, 6.1	5.43, d, 3.2	2.63, dd, 16.1, 1.5	5.05, dd, 8.3, 2.5
4b				2.75, dd, 16.1, 5.5	
4-OH	2.51, br. s				2.89, d, 8.3
6	4.94, br. s	6.84, d, 9.0	4.91, br. s	4.76, d, 2.1	4.60, d, 9.7
7	5.69[b]	5.56, d, 9.0	5.11, br. s	5.17, d, 2.1	5.13, d, 9.7
8-OH	2.67, s		3.15, s	3.15, s	3.42, s
9	5.46[b]	5.22, br. s	5.66, br. s	5.76, br. s	5.41, d, 2.3
10	3.79, br. s	2.92[b]	4.09, br. s	4.12, br. s	2.97, d, 2.3
11		1.97[b]			
12		4.79, br. s			
13	6.03, d, 10.4	1.98[b]	6.04, d, 10.4	6.03, d, 10.1	1.96, d, 8.5
14	6.61, d, 10.4	4.72, br. s	6.71, d, 10.4	6.68, d, 10.1	
15	1.40, s	1.11, br. s	1.14, s	1.08, s	1.57, s
16a	5.69[b]		5.46, br. s	5.12, br. s	5.39, br. s
16b	5.76, br. s		5.56, br. s	5.42, br. s	5.51, br. s
16-OMe		3.82, s			
17	2.48, q, 7.3	2.51, q, 6.8	3.18, q, 7.3	3.08, q, 7.6	3.16, q, 7.3
18	1.24, d, 7.3	1.24, d, 6.8	1.28, d, 7.3	1.31, d, 7.6	1.18, d, 7.3
20	1.40, s	1.12, d, 7.3	1.36, s	1.37, s	1.38, s
2-Ac		1.90, s			
4-Ac			2.08, s		
9-Ac	2.15, s	2.19, s	2.17, s		2.19, s
11-Ac	1.96, s		2.07, s	2.07, s	2.00, s
12-Ac		2.02, s			
14-Ac		1.94, s			
2′a		2.43[b]		4.22, dd, 16.2, 2.5	
2′b		2.46[b]		4.27, dd, 16.2, 2.5	
2′-OH				2.25, t, 2.5	
3′		4.16, m			
4′		1.22, d, 7.3			

[a] Spectra were recorded in $CDCl_3$ (in ppm, multiplicity, *J* in Hz). [b] Overlapped with other signals.

only by the nature of the acyl group at C-4 (a, 3-acetoxybutanoyl in **12** in place of the 3-hydroxybutanoyl in **6**). This relationship was proven by standard acetylation of erythrolide S (**6**) which afforded erythrolide J (**12**), identical by NMR and $[\alpha]_D$ comparison to the authentic material isolated from the same extract. The chemical interconversion of **6** and **12** shows that erythrolide S has the same relative stereochemistry reported for the diterpenoid core of **12**. Furthermore, the presence of a free hydroxyl at C-3′ in **6** provided the opportunity to apply Mosher methodology to determine the C-3′ absolute stereochemistry, which was left undetermined in erythrolide J. Two aliquots of **6** were treated with (−)- and (+)-MTPA chloride in dry pyridine providing the *S* (**6a**) and *R* (**6b**) MTPA esters, respectively. The obtained distribution of $\Delta\delta(S-R)$ values, reflecting the anisotropic effect of MTPA in accordance with the Mosher model, indicated the 3′S configuration. Among the many known briarane diterpenoids isolated from several sources, erythrolide S (**6**) is the first molecule to bear a 3-hydroxybutanoyl substituent.

Two additional new briarane diterpenes, erythrolides T (**7**) and U (**8**), both have a 2,3-epoxide moiety. Erythrolide T (**7**), isolated as a colorless glass, gave a $[M + NH_4]^+$ ion at *m/z* = 572.1899 in the HRCIMS compatible with the molecular formula $C_{26}H_{31}ClO_{11}$ (calcd. for $C_{26}H_{35}{}^{35}ClNO_{11}$; 572.1899), possessing only an oxygen atom more than the molecular formula of erythrolide B (**2**). Comparison of the ^{1}H and ^{13}C NMR spectra of **7** (Tables 1 and 2, respectively) with those reported for erythrolide B confirmed the close structural similarity of these two metabolites. Indeed, resonances assigned in **7** to the entire structural framework going from C-4 to C-14, including the γ-lactone system and the six-membered ring, appear almost identical to those of the corresponding centers of erythrolide B (**2**). On the other hand, in both the ^{1}H and ^{13}C NMR spectra of **7**, resonances for a $\Delta^{2,3}$ double bond are absent, being replaced by signals attributable to epoxide methines (δ_H = 3.24, bs and 3.69 ppm, bd; δ_C = 64.2 and 55.2 ppm, respectively). A detailed analysis of the 2D NMR spectroscopic data (COSY, HMQC, and HMBC, see Figure 1) allowed us to unambiguously locate the epoxide group at C-2/C-3 [COSY: cross peak δ = 3.69 (3-H)/δ = 5.43 (4-H); HMBC: cross-peaks 2-H (δ = 3.24 ppm)/C-15 (17.5) and C-4 (δ = 69.5 ppm)], confirming the assignment of erythro-

Table 2. ^{13}C NMR (100 MHz) data for erythrolides R−V (**5**−**9**) in $CDCl_3$

Pos.	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]
1	41.6, s	45.5, s	40.2, s	40.6, s	36.6, d
2	142.5, d	73.8, d	64.2, d	62.2, d	123.4, d
3	136.2, d	36.9, t	55.2, d	54.0, d	138.0, d
4	73.3, d	68.2, d	69.5, d	37.1, t	76.9, d
5	144.9, s	137.8, s	140.4, s	137.3, s	142.1, s
6	66.1, d	137.9, d	67.3, d	67.8, d	60.3, d
7	79.1, d	76.3, d	79.5, d	78.6, d	80.3, d
8	80.5, s	82.6, s	80.5, s	80.0, s	83.2, s
9	77.8, d	75.8, d	80.2, d	80.3, d	80.7, d
10	49.2, d	33.6, d	43.2, d	41.6, d	43.2, d
11	81.1, s	43.6, d	82.1, s	81.4, s	88.0, s
12	195.6, s	72.8, d	196.1, s	194.5, s	204.5, s
13	125.9, d	24.0, t	126.1, d	124.4, d	39.7, d
14	155.0, d	74.3, d	153.0, d	153.6, d	29.3, s
15	23.4, q	15.0, q	17.5, q	16.4, q	22.6, q
16	115.4, t	169.0, s	118.1, t	119.1, t	125.3, t
16-OMe		52.8, q			
17	44.8, d	43.4, d	45.5 d	45.8 d	43.6, d
18	9.3, q	6.4, q	9.2, q	9.5, q	9.4, q
19	176.3, s	175.3, s	175.2, s	175.6, s	175.1, s
20	20.7, q	15.2, q	21.0, q	21.6, q	22.2, q
2-Ac		21.0, q 171.0, s			
4-Ac			21.5, q 170.2, s		
9-Ac	21.1, q 169.5, s	21.2, q 171.1, s	20.2, q 170.0, s		21.7, q 172.4, s
11-Ac	21.2, q 171.1, s		21.1, q 171.1, s	21.3, q 169.0, s	21.2, q 168.9, s
12-Ac		21.4, q 171.2, s			
14-Ac		21.6, q 169.9, s			
1′		173.0, s		171.8, s	
2′		43.2, t		61.0, t	
3′		64.6, d			
4′		22.8, q			

[a] Assignments made by HMQC and HMBC experiments; in ppm, multiplicity.

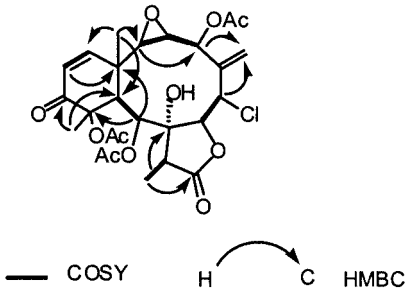


Figure 1. COSY and HMBC correlations observed for erythrolide T (**7**)

lide T (**7**) as the 2,3-epoxide derivative of erythrolide B (**2**). The ROESY spectrum of **7** (Figure 2) was used to infer the relative stereochemistry of this new erythrolide. In particular, as predicted on the basis of close similarities in the ^1H (both δ and *J*) and ^{13}C NMR spectra of **7** and **2**, all the NOE interactions observed for the structural fragment going from C-4 to C-14 of erythrolide T (**7**) appeared consistent with the stereochemistry reported for the same region in erythrolide B (**2**). Moreover, the spatial proximities H-2/H-10 and H-3/Me-15 indicated by NOEs defined the relative orientation of the epoxide ring, thus completing the stereostructure of erythrolide T (**7**) as shown.

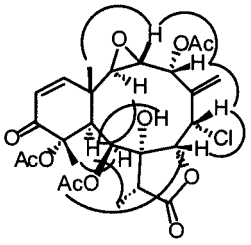
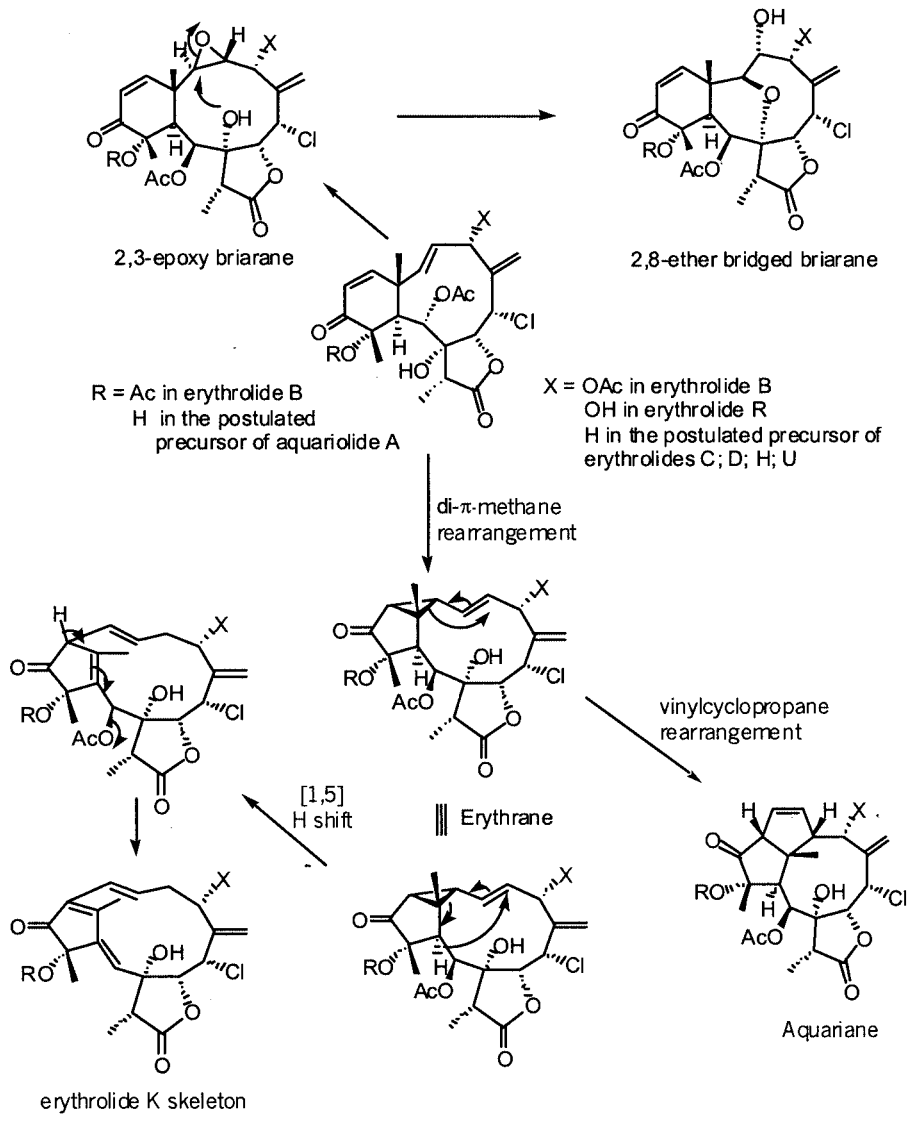


Figure 2. ROESY correlations observed for erythrolide T (**7**)

The isolation of erythrolide T (**7**) is particularly interesting because this molecule is the first erythrolide to possess simultaneously a 2,3-epoxide and an acetate functionality at C-4.[7] Since the 2,3 epoxide molecules are believed to be the precursors of C-2/C-8 ether-bridged derivatives (e.g. erythrolides E, F, G, I; see Scheme 1), it seems reasonable to presume that 4-acetoxy-C-2/C-8 ether erythrolides are also part of the diterpenoid pool of *E. caribaeorum*, but most likely they have never been isolated because of their limited concentrations.

Parallel analysis of the spectroscopic data obtained for erythrolide U (**8**), a colorless glass with a molecular formula of $C_{24}H_{29}ClO_{10}$ (HRCIMS: $[M + NH_4]^+$ *m/z* = 530.1789, calcd. for $C_{24}H_{33}{}^{35}ClNO_{10}$; 530.1793), and those reported for erythrolide D (**13**)[2b] revealed a very close similarity between these two metabolites. In particular, the ^1H and ^{13}C NMR assignments for erythrolide U (Tables 1 and 2, respectively) for the entire diterpenoid core are almost identical to those reported for erythrolide D. The only differences in the two series of data are the lack of H/C signals accounting for one acetyl group and the upfield shift of 2′-H_2 (δ = 4.22 and 4.27 ppm instead of δ = 4.52 and 4.62) in the ^1H NMR spectrum of **8**. Standard acetylation (Ac_2O/pyr) of **8** afforded quantitatively erythrolide D (**13**), demonstrating that erythrolide U is the 2′-desacetyl derivative of erythrolide D (**13**). Interestingly, compound **8** is the putative epoxide precursor of erythrolide I, a C-2/C-8 ether-bridged molecule reported by Schmitz et al.[2b]

Erythrolide V (**9**)[8] was isolated as a white amorphous solid from the more polar chromatography fractions. The HRCIMS ($[M + NH_4]^+$ *m/z* = 514.1850, calcd. for $C_{24}H_{33}{}^{35}ClNO_9$; 514.1844) and ^{13}C NMR spectroscopic data (Table 2) established the molecular formula $C_{24}H_{29}ClO_9$ for **9**, which is identical to the formula of erythrolide R (**5**). Analysis of the 1D and 2D ^1H (Table 1) and ^{13}C NMR spectroscopic data obtained for **9** revealed the presence of a cyclopropane ring (13-H: δ = 1.96, C-13: δ = 39.7; 1-H: δ = 2.37, C-1: δ = 36.6; C-14: δ = 29.3 ppm) fused with a ketone-containing (δ_C = 204.5 ppm) five-membered ring. COSY and HMBC correlations indicated that the remaining carbon of the three-membered ring must be directly connected to a double bond (δ_H = 6.37 and 5.98



Scheme 1. Proposed biogenetic interconversions of *E. caribaeorum* diterpenoids

ppm; δ_C = 123.4 and 138.0 ppm, respectively), which is part of an eleven-membered ring. These structural features are typical of the tricyclo[8.3.1.0^{13,14}]tetradecane system of erythrane diterpenoids. To date, only two molecules possessing such a skeleton have been reported in the literature, namely erythrolides A (**1**)[2a] and L.[2f] The NMR spectroscopic data obtained for erythrolide V (**9**) appeared very similar to those reported for erythrolide A (**1**),[2a] differing only in the lack of one set of acetyl resonances and the upfield shift of the 4-H resonance (δ = 5.05 instead of δ = 5.99 ppm) in the ^{1}H NMR spectrum of **9**. Taken together, this evidence indicates that erythrolide V (**9**) is the 4-desacetyl derivative of erythrolide A (**1**). Acetylation of the secondary alcohol (Ac_2O/pyr) of erythrolide V (**9**) afforded a compound identical (NMR and $[\alpha]_D$) to erythrolide A (**1**), which proved this relationship and completely defined the stereostructure of erythrolide V as reported in **9**. It should be noted that compound **9** is the expected product resulting from the di-π-methane rearrangement of erythrolide R (**5**), described above.

Examination of the diterpenoid fraction of wild *E. caribaeorum* confirmed the presence of aquariolide A (**4**), previously obtained from the cultured material, and also afforded aquariolides B (**10**) and C (**11**), two additional aquariane diterpenes. Structure elucidation of aquariolide B (**10**), a colorless glass with a molecular formula of $C_{25}H_{31}ClO_9$ (HRCIMS: $[M + NH_4]^+$ *m/z* = 528.2001, calcd. for $C_{25}H_{35}{}^{35}ClNO_9$; 528.2000), relied upon detailed analysis of 1D and 2D ^{1}H and ^{13}C NMR spectroscopic data (Table 3). In particular, a series of cross-peaks in the COSY spectrum, identified a ^{1}H spin system extending from the deshielded methine 13-H (δ_H = 3.29, δ_C = 69.8 ppm) to the deshielded methine 4-H (δ_H = 5.70, δ_C = 68.0 ppm), encompassing the *cis*-double bond methines 1-H/2-H. Simple vicinal moieties constitute the remaining three ^{1}H spin systems, namely 6-H/7-H, 9-H/10-H, and 17-H/18-H. Furthermore, five uncoupled methyls (δ = 1.04, 1.59, 3.10 and the acetyl singlets at δ = 2.08 and 2.19 ppm), one OH singlet (δ = 2.79 ppm), and two olefinic methylene protons (δ = 6.00 and 5.98 ppm, long-range coupled with both 6-H and

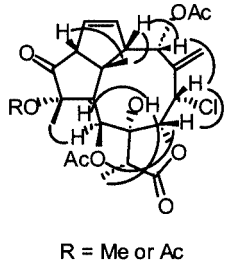
Table 3. ^{1}H (500 MHz) and ^{13}C NMR (100 MHz) data for aquariolides B−C (**10**−**11**)

Pos.	**10**[a]		**11**[a]	
	δ_H, mult., *J*	δ_C, mult.	δ_H, mult., *J*	δ_C, mult.
1	6.08, m	131.3, d	6.14, m	130.4, d
2	5.81, ddd, 7.0, 2.7, 2.0	126.9, d	5.91, ddd, 7.0, 2.7, 2.0	127.5, d
3	3.01, m	63.2, d	3.08, m	64.4, d
4	5.70, br. d, 3.0	68.0, d	5.70, d, 4.0	67.6, d
5		140.1, s		137.6, s
6	5.29, ddd, 4.7, 2.0, 1.8	62.1, d	5.22, ddd, 4.7, 2.0, 1.8	61.4, d
7	5.44, d, 4.7	76.4, d	5.43[a]	76.2, d
8		82.3, s		82.7, s
8-OH	2.79, s		2.70, s	
9	5.66, d, 3.5	67.1, d	5.43[b]	67.9, d
10	2.69, d, 3.5	44.7, d	3.91, d, 3.4	38.7, d
11		83.6, s		84.4, s
11-OMe	3.10, s	51.1, q		
12		210.1, s		208.6, s
13	3.29, m	69.8, d	3.25, m	68.4, d
14		49.3, s		46.1, s
15	1.59, s	29.5, q	1.46, s	28.5, q
16a	5.98, br. s	123.4, t	6.06, d, 2.0	125.2, t
16b	6.00, br. d, 2.0		6.31, d, 1.8	
17	2.51, q, 7.3	43.1, d	2.38, q, 7.2	43.2, d
18	1.20, d, 7.3	6.9, q	1.16, d, 7.2	6.5, q
19		174.9, s		174.6, s
20	1.04, s	14.8, q	1.41, s	19.9, q
4-Ac	2.08, s	21.1, q	2.11, s	21.2, q
		170.7, s		170.9, s
9-Ac	2.19, s	21.6, q	2.25, s	21.6, q
		168.7, s		169.4, s
11-Ac			1.89, s	20.9, q
				169.0, s

[a] Spectra were recorded in $CDCl_3$ (in ppm, multiplicity, *J* in Hz for ^{1}H data). [b] Overlapped with other signals

4-H), are the other signals appearing in the ^{1}H NMR spectrum of **10**. The ^{13}C NMR spectrum of **10** (Table 3) shows resonances for the seventeen protonated carbons, assigned from the HMQC spectrum, and also signals for eight quaternary carbons. The quaternary carbon signals are attributable to two acetyl carbonyls (δ = 170.7 and 168.7 ppm), lactone (δ = 174.9 ppm) and ketone (δ = 210.1 ppm) carbonyls, a double-bond carbon (δ = 140.1 ppm), and three sp^3 carbon atoms (δ = 49.3, 82.3, and 83.6 ppm). The connection of all the above partial structures, which was accomplished by analysis of HMBC correlations and facilitated by comparison with data reported for aquariolide A (**4**),[6] led to a tricyclic skeleton typical of the aquariane framework. In accordance with the molecular formula, aquariolide B (**10**) only differed from aquariolide A (**4**) by the presence of a methyoxy group in place of a hydroxy group. The methoxy substituent was unequivocally located at C-11 on the basis of the HMBC cross-peak between the methyl singlet at δ = 3.10 and the resonance assigned to the oxygenated carbon C-11 (δ = 83.6 ppm). ROESY data (Figure 3) showed that the relative stereochemistry of aquariolide B (**10**) is identical to that reported for aquariolide A (**4**).

A similar analysis was undertaken for the structure elucidation of aquariolide C (**11**), a colorless glass with a molecular formula of $C_{26}H_{31}O_{10}Cl$ (HRCIMS: $[M + H]^+$ *m/z* = 539.1692, calcd. for $C_{26}H_{32}{}^{35}ClO_{10}$; 539.1684). Comparison



R = Me or Ac

Figure 3. ROESY correlations observed for aquariolides B (**10**) and C (**11**)

of the spectroscopic data obtained for **11** with those of aquariolide A (**4**), revealed that the NMR spectra for aquariolide C (**11**) only differ by the presence of additional C/H resonances (Table 3) ascribable to an additional acetyl group. 2D NMR experiments showed that the additional acetyl must be linked to the quaternary carbon C-11 making aquariolide C (**11**) the 11-*O*-acetyl derivative of aquariolide A. Analysis of the ROESY data obtained for **11** (Figure 3) revealed that **4** and **11** share the same relative stereochemistry.

The two new members of the aquariane family (**10** and **11**) differ from the previously reported aquariolide A (**4**) only in the group linked to the oxygen atom at C-11. In particular, aquariolide C (**11**) can be considered to be the direct product of the vinyl−cyclopropane rearrangement of

erythrolide A (**1**), one of the most abundant secondary metabolites of *E. caribaeorum*. However, it should be noted that the major aquariane diterpene in this organism is not aquariolide C (**11**) but the C-11 OH analog aquariolide A (**4**), which is about ten times more abundant than aquariolide C. The relative concentrations of **4** and **11** would seem to rule out a simple chemical conversion of **1** to **11** and instead argues for enzymatic involvement in the conversion of erythranes (i.e. **1**) into aquarianes (i.e. **11**).[6]

Aquariolide B (**10**) is noteworthy since it is the first diterpene from *E. caribaeorum* to bear a methoxy group. In addition, to the best of our knowledge, there are no other reports of methoxy-substituted briarane or briarane-related diterpenes from octocorals.[1] Consequently, the presence of the unusual OMe group raised the possibility that it might have been formed from MeOH during the extraction/purification steps. To resolve this issue, a small piece (ca. 200 g wet weight) of wild *E. caribaeorum* was extracted, and the extract fractionated, by replacing MeOH with EtOH in all the steps. After fractionation of the organic extract according to the scheme described above, a fraction was obtained that contained aquariolide B (**10**) by NMR and TLC analysis. Furthermore, erythrolide A (**1**) and aquariolide A (**4**) were dissolved in MeOH and left at room temp. for several weeks but this led only to the recovery of intact starting material. Taken together, this evidence suggests an enzymatic origin for the methoxy group of aquariolide B (**10**).

Gorgonians are known to contain a wide array of diterpenoid derivatives mostly belonging to the cembrane, asbestinane, pseudopterosane, eunicellane, and briarane classes.[1] Among them, erythrolide B (**2**) must be considered quite remarkable. This highly oxygenated briarane diterpene is the major metabolite of *E. caribaeorum* (about 30% of the organic extract and 0.5% of wet weight in the specimen examined in this work) and, most likely, it plays a key role in the biogenetic origin of many of the diterpenoids of this organism. Scheme 1 presents a summary of the structural diversity of diterpenoids isolated from *E. caribaeorum*, that can be related to erythrolide B (**2**). The key sites of reactivity in erythrolide B are the two double bonds $\Delta^{2,3}$ and $\Delta^{13,14}$. Epoxidation at $\Delta^{2,3}$ produces the first series of derivatives, which, as a result of nucleophilic attack of 8-OH at C-2, gives rise to the C-2/C-8 ether-bridged molecules. Moreover, the 1,4-diene system in **2** is the substrate for the di-π-methane rearrangement yielding the erythrane skeleton found in erythrolide A (**1**) (and also in erythrolides L and V). Interestingly, the erythrane system possesses another structural moiety, the vinylcyclopropane, that can undergo further rearrangements. The most important reactions reported for the vinylcyclopropane moiety are the [1,5]-H shift and the so-called vinyl−cyclopropane rearrangement (VCR). The direct product of a [1,5]-H shift in erythrolide A (**1**) has never been found. However, Reynolds et al.[2d] have proposed that the [1,5]-H shift product of erythrolide A is too labile to isolate and, most likely, erythrolide K is an artifact formed from it by elimination of acetic acid during chromatography on silica gel. The isolation of aquariolide C (**11**), the product of a VCR of erythrolide A (**1**), completes the picture.

It is not clear whether this series of rearrangements is entirely photochemical or enzyme mediated. Although it is possible to reproduce the di-π-methane rearrangement of erythrolide B (**2**) and a [1,5]-H shift in erythrolide A (**1**) in the laboratory, the involvement of enzymes in these reactions in the living organism cannot be excluded. On the other hand, the parallel observations that photochemical irradiation of erythrolide A (**1**) does not produce aquariolide C (**11**)[6] and that the major aquariane of *E. caribaeorum*, aquariolide A (**4**), is not the product of direct rearrangement of erythrolide A (**1**), the major erythrane, both strongly suggest that the erythrane to aquariane conversion is enzymatic. Of course, this matter will require further study. The possibility of isolating enzymes responsible for catalyzing these di-π-methane, [1,5]-H shift, and VCR reactions, for which there is no precedent in nature, is particularly intriguing.

Several of the diterpenes described above, which were obtained in sufficient amounts for testing, have been subjected to in vitro cytotoxicity assays and the results are shown in Figure 4. Erythrolides P (**14**)[2f] and J (**12**) show significant cytotoxic activity with IC_{50}'s less than 4 μg/mL, while erythrolide T (**13**) showed no activity at the concentrations tested, and erythrolide D (**13**) had an intermediate potency. Aquariolides B (**10**) and C (**11**) showed activity similar to erythrolide D (**13**). It is interesting to note that both erythrolides P (**14**) and J (**12**) have fully reduced cyclohexane rings with either acetoxy or hydroxy functionalities at both C-12 and C-14, suggesting that this substructure might be important for maximum cytotoxicity in this family of compounds.

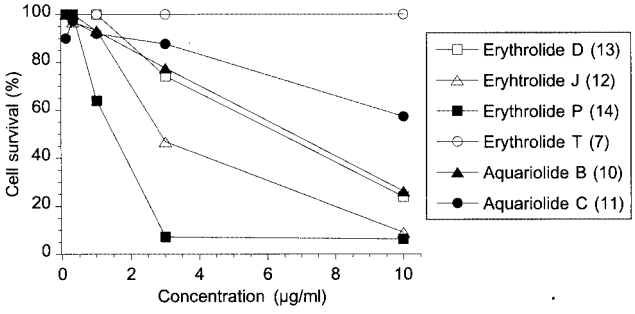


Figure 4. In vitro cytotoxicity of *E. caribaeorum* diterpenoids against human breast cancer MCF-7 cells

Experimental Section

General Experimental Procedures: IR spectra (KBr) were recorded on a Bruker model IFS-48 spectrophotometer. UV spectra were obtained in MeOH using a Beckman DU70 spectrophotometer. Low- and high-resolution CI mass spectra were recorded on a Kratos mass spectrometer. ^1H (500 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker AMX-500 and AM-400 spectrometers, respectively. NMR chemical shifts are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.24, δ_C = 77.0 ppm). One bond heteronuclear connectivities were determined with HMQC pulse sequence using a BIRD pulse of 0.50 s (interpulse

delay set for $^1J_{C,H}$ = 135 Hz). Two- and three-bond ^1H-^{13}C connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 10 Hz. HPLC separations were achieved on a Waters apparatus equipped with Alltech columns (250 × 4.6 mm) and monitored by a RI detector.

Animal Material, Extraction, and Isolation: Specimens of *Erythropodium caribaeorum* were collected in the Summer 2001 during an expedition to Dominica, immediately frozen, and transported to Vancouver at 0 °C. Thawed samples (4.8 kg wet wt.) were cut into small pieces and extracted multiple times with MeOH. The combined MeOH extracts were concentrated to a brown-colored gum in vacuo which was then partitioned between ethyl acetate (EtOAc) and water. The organic layer was subsequently partitioned between *n*-hexane and MeOH/H_2O, 9:1, yielding 32.5 g of this latter phase, which was further fractionated by normal-phase flash chromatography (gradient elution from *n*-hexane/EtOAc, 6:4, to EtOAc to EtOAc/MeOH, 1:1, in 5% increments). Fractions eluted with EtOAc/hexane, 6:4, were further purified by normal-phase HPLC (eluent hexane/EtOAc, 75:25) yielding pure 16-acetylerythrolide H,[2e] and aquariolides B (**10**), and C (**11**). Fractions eluted with EtOAc/hexane, 65:35, were purified by HPLC (eluent hexane/EtOAc, 7:3) yielding pure erythrolides M[2f] and E.[2b] Fractions eluted with EtOAc/hexane, 7:3, were purified by HPLC (eluent hexane/EtOAc, 65:35) yielding pure aquariolide A (**4**)[6] and erythrolide T (**7**). Fractions eluted with EtOAc/hexane, 8:2, contained pure erythrolide B(**2**),[2a] and, analogously, those eluted with EtOAc/hexane, 9:1, contained pure erythrolide A (**1**).[2a] Fractions eluted with EtOAc/hexane, 95:5, were purified by HPLC (eluent EtOAc/hexane, 75:25) yielding pure erythrolides Q,[2f] J (**12**),[2c] U (**8**), and V (**9**). Fractions eluted with EtOAc were purified by HPLC (eluent EtOAc/hexane, 8:2) yielding pure erythrolides R (**5**) and S (**6**). Fractions eluted with EtOAc/MeOH 95:5 were purified by HPLC (eluent EtOAc/hexane, 9:1) yielding pure erythrolide H.[2b] Fractions eluted with EtOAc/MeOH 8:2 contained pure erythrolide P.[2f]

Erythrolide R (5): Pale yellow oil, 23.2 mg. $[\alpha]_D^{25}$ = +15.4 (*c* = 2.4 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3550, 1780, 1740 cm^{-1}. UV (MeOH): λ_{max} = 216 (ε 7500) nm. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2. CIMS (positive ions): *m/z* = 497, 499 (≈ 3:1) $[M + H]^+$. HRCIMS: *m/z* = 497.15790, calcd. for $C_{24}H_{30}{}^{35}ClO_9$, *m/z* = 497.15784.

Erythrolide S (6): Pale yellow amorphous solid, 3.2 mg. $[\alpha]_D^{25}$ = +111.0 (*c* = 1.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3445, 1777, 1736 cm^{-1}. UV (MeOH): λ_{max} = 224 (ε 3150). ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2. CIMS (positive ions): *m/z* = 716 $[M + NH_4]^+$. HRCIMS: *m/z* = 716.31264, calcd. for $C_{33}H_{50}NO_{16}$, *m/z* = 716.31296.

Preparation of (*S*)- and (*R*)-MTPA Esters of Erythrolide S (6): Erythrolide S (**6**) (0.8 mg) was dissolved in 300 μL of dry pyridine, treated with (−)-MTPA chloride (10 μL) and then stirred overnight at room temp. After removal of the solvent in high vacuum, the obtained solid contained the (*S*)-MTPA ester **6a**. Using (+)-MTPA chloride, the same procedure afforded the (*R*)-MTPA ester **6b**.

Compound 6a [(*S*) MTPA ester]: FABMS: *m/z* = 915 $[M + H]^+$. HRFABMS: *m/z* = 915.3270, calcd. for $C_{43}H_{54}F_3O_{18}$ 915.3262. ^1H NMR ($CDCl_3$): δ = 7.37 and 7.47 (m, MTPA phenyl protons), 6.87 (bd, *J* = 10.0 Hz, 6-H), 5.82 (dd, *J* = 12.4, 5.8 Hz, 4-H), 5.58 (d, *J* = 10.0 Hz, 7-H), 5.56 (m, 3′-H), 5.25 (br. s, 9-Hs), 4.92 (d, *J* = 8.5 Hz, 2-H), 4.79 (d, *J* = 2.0 Hz, 12-H), 4.71 (d, *J* = 3.1 Hz, 14-H), 3.75 (s, OMe-16), 3.57 (s, MTPA-O*Me*), 3.05 (d, *J* = 4.0 Hz, 10-H), 2.91 (m, 3a-H), 2.89 (dd, *J* = 11.4, 7.5 Hz, 2′a-H), 2.69 (dd, *J* = 11.4, 9.9 Hz, 2′b-H), 2.56 (q, *J* = 7.1 Hz, 17-H), 2.19 (s, CH_3COO-9s), 2.04 (s, 12-CH_3COO), 1.96 (3b-H, 11-H, 13-H_2, overlapped), 1.93 (s, 14-CH_3COO), 1.89 (s, 2-CH_3COO), 1.26 (d, *J* = 7.1 Hz, 4′-H_3), 1.23 (d, *J* = 7.1 Hz, 18-H_3), 1.12 (s, 15-H_3), 1.11 (d, *J* = 7.6 Hz, 20-H_3).

Compound 6b [(*R*) MTPA ester]: FABMS: *m/z* = 915 $[M + H]^+$. HRFABMS: *m/z* = 915.3268, calcd. for $C_{43}H_{54}F_3O_{18}$ 915.3262. ^1H NMR ($CDCl_3$): δ = 7.33 and 7.54 (m, MTPA phenyl protons), 6.87 (bd, *J* = 10.0 Hz, 6-H), 5.82 (dd, *J* = 12.4, 5.8 Hz, 4-H), 5.58 (d, *J* = 10.0 Hz, 7-H), 5.56 (m, 3′-H), 5.23 (br. s, 9-H), 4.88 (d, *J* = 8.5 Hz, 2-H), 4.81 (d, *J* = 2.0 Hz, 12-H), 4.73 (d, *J* = 3.1 Hz, 14-H), 3.88 (s, 16-OMe), 3.60 (s, MTPA-O*Me*), 2.95 (d, *J* = 4.0 Hz, 10-H), 2.91 (m, 3a-H), 2.75 (dd, *J* = 11.4, 7.5 Hz, 2′a-H), 2.56 (q, *J* = 7.1 Hz, 17-H), 2.36 (dd, *J* = 11.4, 9.9 Hz, 2′b-H), 2.20 (s, 9-CH_3COO), 2.04 (s, 12-CH_3COO), 1.96 (3b-H, 11-H, 13-H_2, overlapped), 1.95 (s, 14-CH_3COO), 1.92 (s, 2-CH_3COO), 1.32 (d, *J* = 7.1 Hz, 4′-H_3), 1.23 (d, *J* = 7.1 Hz, 18-H_3), 1.12 (s, 15-H_3), 1.11 (d, *J* = 7.6 Hz, 20-H_3).

Application of the Mosher's Method to the 3-Hydroxybutanoyl Moiety of 6: Δδ(*S*−*R*): +0.14 ppm (2′a-H); +0.33 ppm (2′b-H); −0.06 ppm (4′-H_3). These differences imply the *S* configuration at C-3′.

Erythrolide T (7): Colorless glass, 1.3 mg. $[\alpha]_D^{25}$ = +25.7 (*c* = 1.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3540, 1780, 1733 cm^{-1}. UV (MeOH): λ_{max} = 214 (ε 8965) nm. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2. CIMS: (positive ions): *m/z* = 572, 574 (≈ 3:1) $[M + NH_4]^+$. HRCIMS: *m/z* = 572.18993, calcd. for $C_{26}H_{35}{}^{35}ClNO_{11}$, *m/z* = 572.18987.

Erythrolide U (8): Colorless glass, 1.2 mg. $[\alpha]_D^{25}$ = −35.1 (*c* = 1.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3542, 1775, 1743 cm^{-1}. UV (MeOH): λ_{max} = 216 (ε 6500). ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2. CIMS: (positive ions): *m/z* = 530, 532 (≈ 3:1) $[M + NH_4]^+$. HRCIMS: *m/z* = 530.17885, calcd. for $C_{24}H_{33}{}^{35}ClNO_{10}$, *m/z* = 530.17930.

Erythrolide V (9): White amorphous solid, 3.4 mg. $[\alpha]_D^{25}$ = −38.0 (*c* = 1.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3543, 1769, 1745 cm^{-1}. UV (MeOH): λ_{max} = 215 (ε 8500) nm. ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2. CIMS (positive ions): *m/z* = 514, 516 (≈ 3:1) $[M + NH_4]^+$. HRCIMS: *m/z* = 514.18495, calcd. for $C_{24}H_{33}{}^{35}ClNO_9$, *m/z* = 514.18439.

Aquariolide B (10): Colorless glass, 4.5 mg. $[\alpha]_D^{25}$ = −62.5 (*c* = 1.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3430, 1775, 1737 cm^{-1}. UV (MeOH): λ_{max} = 213 (ε 3800) nm. ^1H and ^{13}C NMR ($CDCl_3$): Table 3. CIMS (positive ions): *m/z* = 528, 530 (≈ 3:1) $[M + NH_4]^+$. HRCIMS: *m/z* = 528.20005, calcd. for $C_{25}H_{35}{}^{35}ClNO_9$, *m/z* = 528.20004.

Aquariolide C (11): Colorless glass, 1.1 mg. $[\alpha]_D^{25}$ = −167.2 (*c* = 2.0 mg/mL). IR (KBr): $\tilde{\nu}_{max}$ = 3432, 1776, 1740 cm^{-1}. UV (MeOH): λ_{max} = 213 (ε 3500) nm. ^1H and ^{13}C NMR ($CDCl_3$): Table 3. CIMS (positive ions): *m/z* = 539, 541 (≈ 3:1) $[M + H]^+$. HRCIMS: *m/z* = 539.16918, calcd. for $C_{26}H_{32}{}^{35}ClO_{10}$, *m/z* = 539.16840.

Acetylation of Erythrolides R (5), S (6), U (8), and V (9): Erythrolide R (**5**) (3.5 mg) was dissolved in 1.0 mL of dry pyridine and 1.0 mL of acetic anhydride and the resulting mixture was stirred at room temp. overnight. The reaction solvents were removed by evaporation under high vacuum and purification of the product by normal phase HPLC yielded 2.9 mg of erythrolide B (**2**). Application of the same procedure to: a) erythrolide S (**6**, 0.8 mg) yielded 0.5 mg of erythrolide J (**12**); b) erythrolide U (**8**, 0.6 mg) yielded

0.5 mg of erythrolide D (**13**); c) erythrolide V (**9**, 1.5 mg) yielded 1.0 mg of erythrolide A (**1**). The obtained erythrolides B, J, D, and A were identified by comparison of their spectroscopic data with those reported in the literature.

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