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Cyclobutenbriarein A, the First Diterpene with a Tricyclo[8.4.0.0^{3,6}]tetradec-4-ene Ring System Isolated from the Gorgonian *Briareum asbestinum*

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Received August 9, 2001

Cyclobutenbriarein A (**2**), a novel class of C20-rearranged diterpene briarane possessing an unprecedented tricyclo[8.4.0.0^{3,6}]tetradec-4-ene ring system was isolated, along with five new briarane diterpenoids (**1** and **3**−**6**), from the organic extracts of the Bahamian gorgonian *Briareum asbestinum*. The structures of these secondary metabolites were established on the basis of extensive NMR studies and accurate mass measurements (HRFABMS).

Introduction

Marine gorgonians have been shown to produce a large variety of terpene metabolites that are largely unknown in terrestrial sources.[1,2] Specimens belonging to the genus *Briareum* (syn: *Solenopodium*, Briareidae family)[3] have been subjected to numerous chemical investigations yielding a wide variety of diterpenoids, most belonging to the oxygenated 2,11-cyclized cembranoid family.[4] The gorgonian *Briareum asbestinum*, an exceedingly variable species commonly distributed from southern Florida through most of the West Indies, has proven to be a particularly rich source of this class of diterpenes.[5] The 2,11-cyclized cembranoid diterpenes found in *B. asbestinum* can be grouped into three major carbon skeleton types (oxygenated derivatives): eunicellane (eunicellins/cladiellins and briarellins), asbestinane (asbestinins), and briarane (briarans/briareins) frameworks. In particular, diterpene briareins are characterized by the presence of a γ-lactone fused to a highly substituted bicyclo[8.4.0] ring system. A biosynthetic relationship between the cembrane skeleton and the three carbon skeletons has been proposed by Faulkner.[6]

During the course of our study into metabolites from gorgonians[7] and involving a project addressing the biosynthetic origin of marine terpenes,[8] we have focused our attention on the gorgonian *B. asbestinum*, collected in Bahamas, as it is one of the richest sources of such metabolites. In this paper, we wish to report the structural elucidation of a novel class of C20-rearranged briarane containing an unprecedented tricyclo[8.4.0.0^{3,6}]-tetradec-4-ene ring system, cyclobutenbriarein A (**2**), along with five new briarane diterpenoids (**1** and **3**−**6**, Chart 1).

Results and Discussion

Specimens of *B. asbestinum* were collected by hand using scuba at Sweetings Cay in the Bahamas. Freshly collected animals were frozen on site and transported back to Florida. The specimens were freeze-dried, thawed, and extracted exhaustively with MeOH to obtain an extract that was fractionated using our standard partitioning procedure[9] into several fractions of differing polarity. The CH_2Cl_2 fraction was chromatographed on a silica flash column (CH_2Cl_2 polarized with MeOH) to yield a diterpene mixture which was submitted to repeated normal phase HPLC to give pure compounds **1**−**6**.

The properties of compound **1**, the major component isolated from this gorgonian, was very useful in characterizing the other novel derivatives. The molecular formula of $C_{30}H_{41}ClO_{11}$ for **1** was proposed by the (+)-LRFABMS and (+)-APcIMS pseudomolecular ions at *m*/*z* 635/637 $[M + Na]^+$ and 599 $[M - HCl + Na]^+$ and was verified by subsequent HRFABMS (see the Experimental Section). The diterpene structure of compound **1** was directly inferred from its ^{13}C NMR spectrum where it is possible to account for all 20 carbons after subtraction

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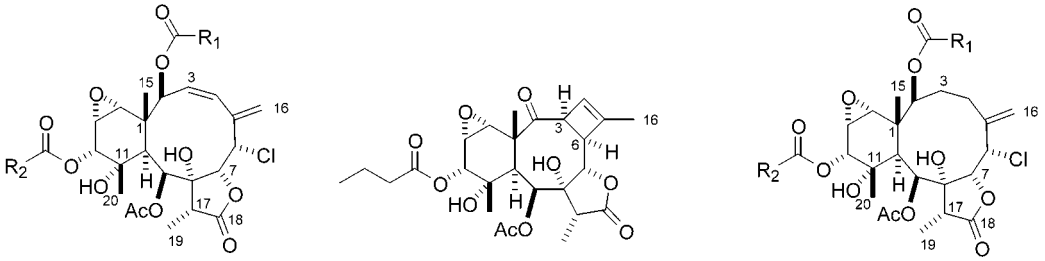
10.1021/jo0160221 CCC: $22.00
Published on Web 06/14/2002

CHART 1



1 R_1=R_2=*n*-Pr
3 R_1=Me, R_2=*n*-Pr
4 R_1=*n*-Pr, R_2=Me

2

5 R_1=R_2=*n*-Pr
6 R_1=Me, R_2=*n*-Pr

of the 10 carbons associated with three ester groups (one acetate and two butyrate groups). The briarein carbon skeleton of this diterpene was initially inferred from the tetrasubstituted carbon atom bearing alkyl substituents at δ_C 43.3 (s) observed in its ^{13}C NMR spectrum, which is characteristic of the carbocyclic ring junction in the briarein series (δ_C 43−46 ppm).[10] The structure of this new briarein was defined by ^1H NMR, ^{13}C NMR, DEPT, and gHMQC experiments. Thus, the presence of an epoxy group in **1** was suggested by the carbon resonances at δ_C 61.9 (d) and 54.0 (d) and it was confirmed by their HMQC correlations to two proton resonances at δ_H 2.92 (1H, d, J = 3.3 Hz) and 3.58 (1H, dd, J = 3.3, 5.8 Hz), respectively. Two hydroxyl groups linked to quaternary carbons were deduced by the ^{13}C NMR signals at δ_C 73.3 (s) and 83.7 (s) and ^1H NMR chemical shifts at δ_H 3.89 (1H, s) and 4.45 (1H, s) assigned to two OH protons. The ^{13}C NMR signals at δc 135.9 (s), 130.0 (d), 128.1 (d), and 117.9 (t), which correlated by HMQC to four olefinic protons at δ_H 6.38 (br s), 5.92 (br s), 5.91 (d, J = 11.3 Hz), and 5.62 (dd, J = 9.3, 11.3 Hz), allowed us to identify a diene system $\Delta^{3,5(16)}$. Finally, the chlorine atom detected in the mass spectrum of **1** was linked to a methine carbon assigned to the resonance at δ_C 63.0 (d), which correlated by HMQC to the methine proton at δ_H 5.18 (br s). gHMBC experiments permitted the connectivity of the isolated proton spin systems deduced from $^1H-^1H$ COSY (Table 1). The comparison of these data to those of other briarein diterpenes reported in the literature indicated that **1** had a structure similar to that of brianthein V, also isolated from *B. asbestinum*, but differed by the presence of an additional OH group at C-11.[11]

The relative stereochemistry of compound **1** was established using information from NOESY correlations (Table 1) and by comparison of its spectroscopic data to those of briarein analogues.[12] A single-crystal X-ray structure analysis was carried out in order to confirm the molecular structure of **1**. Fortunately, X-ray analysis of a suitable crystal of **1** grown from a solution of MeOH furnished the absolute configuration (see the Supporting Information). On the basis of these data, we assigned compound **1** as 11-hydroxybrianthein V and its absolute configuration is 1*R*,2*S*,6*S*,7*R*,8*R*,9*S*,10*S*,11*S*,12*R*,13*R*,-14*R*,17*R*.[13]

Cyclobutenbriarein A (**2**) was isolated as a colorless amorphous solid. The IR spectrum of **2** showed absorptions for ketone (1705 cm^{-1}), ester (1739 cm^{-1}), and lactone (1778 cm^{-1}) carbonyl groups. The pseudomolecular ion $[M + Na]^+$of compound **2** is observed at *m*/*z* 529 in the (+)-LRFAB and (+)-APcI mass spectra. The $[M + H]^+$ and $[M - H_2O + H]^+$ ions are also detected in the APcIMS (+) at *m*/*z* 507 and 489. These values suggested a molecular formula of $C_{26}H_{34}O_{10}$ that was confirmed by HRFABMS of the $[M + Na]^+$ ion at *m*/*z* 529.2046 (Δ 0.4 mmu), which requires 10 degrees of unstauration in the molecule.

The structure of **2** was completely solved by a combination of 1D and 2D NMR methods and by comparison with the NMR data of **1**. The ^{13}C NMR spectrum of **2** displayed 26 carbon resonances, four of them corresponding to four carbonyl groups at δ_C 208.8, 176.3, 174.5, and 169.2 ppm characteristic of a ketone, a lactone, and two ester groups, respectively. One of the ester groups was identified as an acetate by the methyl resonance observed in the ^{13}C and ^1H NMR spectra at δ_C 21.1 (q) and δ_H 2.11 (3H, s), respectively, while the other one was determined to be a butyrate group based on NMR data at δ_C 36.1 (t), 18.5 (t), and 13.6 (q) and δ_H 2.47 (2H, t, J = 7.3 Hz), 1.73 (2H, m), 1.01 (3H, t, J = 7.4 Hz) (see Table 1). Subtraction of the six carbons associated with ester groups left 20 carbons, which were suggestive of a diterpene skeleton. Comparison of the remaining carbon and proton NMR chemical shifts of **2** in relation to those of **1** indicated a rearranged diterpene briarane skeleton.

Seven oxygenated carbon atoms were observed in the ^{13}C NMR spectrum of **2** in the range 51.1−82.9 ppm which were assigned to a γ-lactone, two ester functionalities (one acetate and one butyrate), an epoxy moiety, and two hydroxyl groups linked to quaternary carbons. The resonances of these were very similar to those of **1** and allowed the identification of substructure A but with differences in the chemical shifts at C-1 and C-7 (Figure 1a). These changes, along with the completely different carbon and proton chemical shits between positions C-2 and C-6, indicated that the structures of compounds **1** and **2** differ in that region of the molecule (substructure

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(13) The stereochemistry at C-1 in briareins having a similar substructure around that carbon (i.e., briantheins V, Y, Z and solenolides A and B) was wrongly assigned as 1*S* instead of 1*R* (see ref 10).

TABLE 1. ^{1}H and ^{13}C NMR, GHMBC, and NOESY Spectral Data ($CDCl_3$) of Compounds 1 and 2

	1				2			
C no.	^{13}C	^{1}H	gHMBC	NOESY	^{13}C	^{1}H	gHMBC	NOESY
1	43.3 s				50.3 s			
2	75.8 d	6.22 d, 9.3	C: 1, 3, 4, 10, 14, 15, 2a	H-3, H-16	208.8 s			
3	130.0 d	5.62 dd, 9.3, 11.3		H-2, H_3-15	48.0 d	4.53 dd, 2.3, 4.5		H-6, H-10
4	128.1 d	5.91 d, 11.3	C: 2, 5, 6, 16		127.9 d	5.93 s	C: 3	
5	135.9 s				149.4 s			
6	63.0 d	5.18 br s		H-7	46.5 d	3.76 dd, 4.5, 11.2	C: 3, 4, 5, 7	H-3
7	77.9 d	5.03 d, 4.1		H-6, H-17	82.9 d	4.16 d, 11.2	C: 6, 8	
8	83.7 s				80.1 s			
9	68.7 d	5.78 d, 6.1	C: 7, 8, 10, 11, 17, 9a	H-10, H-17, H_3-19, H_3-20, OH-11	65.7 d	5.55 d, 2.8	C: 7, 8, 10, 11, 9a	
10	36.5 d	2.65 d, 6.1	C: 1, 2, 8, 9, 11, 15, 20	H-2, H-9, OH-8, OH-11	36.2 d	3.16 d, 2.8	C: 1, 2, 8, 9, 11, 15, 20	H-3
11	73.3 s				71.2 s			
12	72.8 d	4.55 d, 5.8	C: 10, 11, 14, 20, 12a	H-13, H-20, OH-11	72.1 d	4.97 d, 4.9	C: 10, 20, 12a	H-13, H_3-20
13	54.0 d	3.58 dd, 3.3, 5.8		H-12, H-14	51.1 d	3.48 dd, 3.6, 4.9	C: 12	H-12, H-14
14	61.9 d	2.92 d, 3.3	C: 1, 10, 15	H-13, H_3-15	59.3 d	2.87 d, 3.6	C: 1, 15	H-13, H_3-15
15	15.5 q	1.18 s	C: 1, 2, 10, 14	H-14, H-3	16.2 q	1.31 s	C: 1, 2, 14	
16	117.9 t	6.38 br s, 5.92 br s	C: 4, 5, 6	H-2	16.6 q	1.85 br s	C: 4, 5, 6	
17	45.7 d	2.39 q, 7.1	C: 8, 9, 18, 19	H-7, H-9, H_3-19	41.9 d	2.23 q, 7.1	C: 18, 19	
18	174.2 s				176.3 s			
19	6.9 q	1.23 d, 7.1	C: 8, 17, 18	H-9, H-17, OH-8, OH-11	6.5 q	1.11 d, 7.1	C: 8, 17, 18	
20	22.3 q	1.30 s	C: 10, 12		25.8 q	1.40 s	C: 10, 11, 12	H-12
OH-8		4.45 s	C: 7, 8, 9			3.22 s		OH-11
OH-11		3.89 s	C: 10, 11, 20			2.64 s		OH-8
ester at C-2[a]	171.9 s							
b	36.1 t	2.29 t, 7.4	C: 21a, 21c					
c	18.3 t	1.67 m	C: 21a, 21b, 21d					
d	13.5 q	0.93 t, 7.4	C: 21b, 21c					
ester at C-9	169.8 s				169.2 s			
b	21.8 q	2.18 s	C: 22a		21.1 q	2.11 s	C: 9a	
ester at C-12	172.8 s				174.5 s			
b	36.3 t	2.34 t, 7.5	C: 23a, 23c		36.1 t	2.47 t, 7.3	C: 12a, 12c, 12d	
c	18.5 t	1.67 m	C: 23a, 23b, 23d		18.5 t	1.73 m	C: 12a, 12b, 12d	
d	13.5 q	0.99 t, 7.5	C: 23b, 23c		13.6 q	1.01 t, 7.4	C: 12b, 2c	

[a] Ester = butyrate or acetate.

B). The six remaining carbon resonances and the tenth unsaturation deduced from the molecular formula of **2** were assigned as follows and allowed us to determine substructure B: (a) the carbonyl group at δ_C 208.8 was indicative of a ketone functionality; (b) two ^{13}C NMR sp^2 signals at δ_C 149.4 (s, C-5) and 127.9 (d, C-4) along with a methyl group at δc 16.6 (q, 16) suggested the presence of a trisubstitued double bond bearing an allylic methyl group in **2**, which was confirmed by the olefinic proton at δ_H 5.93 (s, H-4) and the allylic methyl at δ_H 1.85 (1H, br s, H-16); (c) two methine carbon atoms at δ_C 48.0 (d, C-3) and 46.5 (d, C-6), which correlated by HMQC with proton signals directly connected by ^{1}H$-^1$H COSY at δ_H 4.53 (dd, J = 2.3, 4.5 Hz, H-3) and 3.76 (dd, J = 4.5, 11.2 Hz, H-6), respectively, were located along with the trisubstitued double bond in a cyclobutene ring. Further ^{1}H$-^1$H COSY correlations were observed between allylic methyl H_3-16 and H-3 and H-6 protons.

HMBC correlations allowed us to confirm the identity of substructure B (Figure 1b). Thus, the correlation between the carbonyl group at δ_C 208.8 (s) to H-10 at δ_H 3.16 (d, J = 2.8 Hz) and H-15 at δ_H 1.31 (s) allowed us to locate the ketone functionality at C-2, and this also explained the downfield shift of C-1 at δ_C 50.3 (s) in **2**, in relation to that in **1** (δ_C 43.3). The proton signal at δ_H 3.76 was unambiguously fixed at C-6 position by the ^{1}H$-^1$H COSY correlation with H-7 at δ_H 4.16 (d, J = 11.2 Hz) and the HMBC correlations to carbons C-3, C-4, C-5, and C-7. Furthermore, HMBC key cross-peaks are observed between the proton at δ_H 1.85 (H-16) and C-4, C-5, and C-6. The intriguing cyclobutene ring was confirmed by the inspection of the coupled ^{13}C NMR spectrum of **2**, which displayed the signal at δ_C 127.9 (C-4), as a doublet with a ^1J(CH) of 177 Hz, that resulted larger than the average coupling sp^2 carbon coupling constant.[14]

The ^{1}H NMR coupling constants and NOESY data of compound **2**, and comparison of its spectroscopic data to those of **1**, allowed us to determine the relative stereochemistry of **2**. The same relative configuration of substructure A in compound **2** as in **1** was deduced from the similar carbon and proton chemical shifts and NOESY correlations found in both compounds. In relation to substructure B, the α-orientation for H-3 and H-6 was

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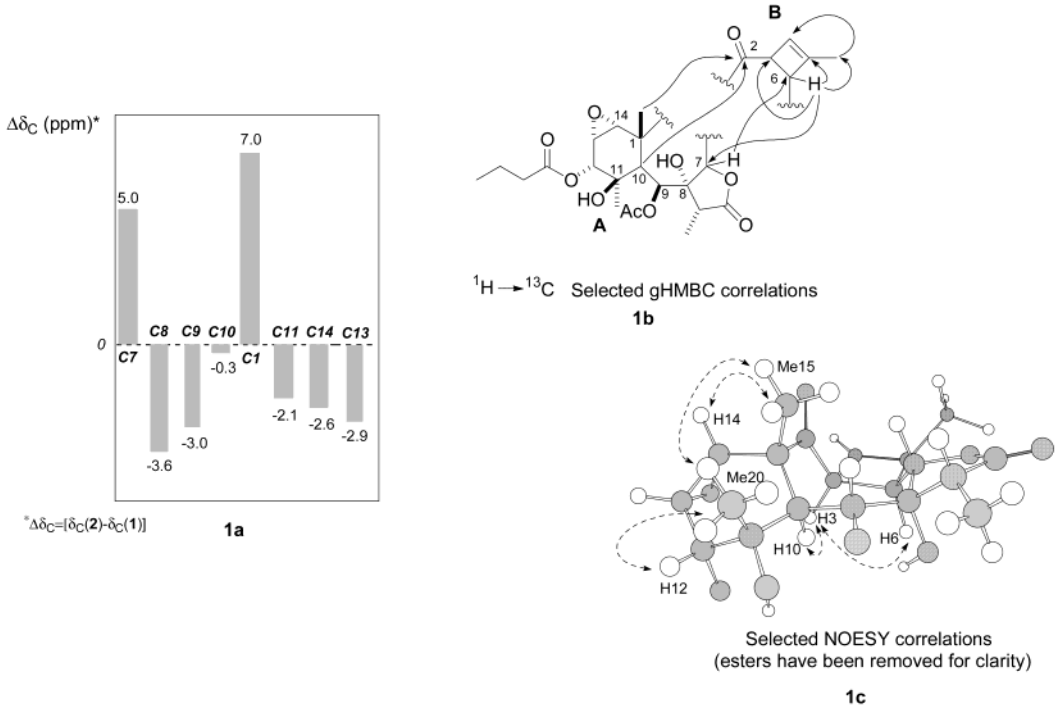


FIGURE 1. (a) Difference between several carbon chemical shifts of cyclobutenbriarein A (**2**) and those of compound **1** (125 MHz, $CDCl_3$). (b) Selected gHMBC correlations of **2**. (c) Selected NOESY correlations of **2**.

suggested from the intense NOESY cross-peaks from H-3 to H-10 and to H-6 (Figure 1c). The almost zero coupling constant observed between H-3 and H-4, the cis coupling constant between H-3 and H-6 (J = 4.5 Hz) in the cyclobutene ring, and the large J value between H-6 and H-7 (J = 11.2 Hz) confirmed the relative α-disposition for H-3 and H-6 in **2**. Thus, the structure of compound **2**, named as cyclobutenbriarein A, was unambiguously established on the basis of these spectral data.

The new briarein diterpene **3** was isolated as a colorless powder. The molecular formula $C_{28}H_{37}ClO_{11}$ was established for compound **3** from (+)-APcIMS showing the pseudomolecular ion $[M + Na]^+$ at m/z 607/609 (confirmed by HRFABMS of the ion at m/z 607.1921, Δ 0.1 mmu) and the base peak at m/z 567/569 $[M - H_2O + H]^+$. The ^{1}H and ^{13}C NMR data of **3** were nearly identical with those of **1** except that the signals corresponding to the propyl group of one of the butyrate esters was missing while an additional acetate signal was observed in the NMR data of **3**. Other fragments observed in the APcIMS of **3** at m/z 419/421 $[M - H_2O - {}^nPrCOOH - AcOH + H]^+$ and m/z 359/361 $[M - H_2O - {}^nPrCOOH - 2AcOH + H]^+$ confirmed the presence of one butyrate and two acetate groups in the molecule. The sites of attachment of the ester groups in the molecule were determined by gHMBC experiments. Thus, the butyrate carbonyl carbon resonance at δ_C 173.0 showed HMBC correlations with both H-12 at δ_H 4.54 (d, J = 5.7 Hz) and the methylene protons of the propyl group at δ_H 1.70 (m) indicating that the butyrate group was located at C-12. On the other hand, the acetate carbonyl carbon resonances at δ_C 170.0 (s) and at δ_C 169.5 (s) displayed HMBC cross-peaks with H-9 at δ_H 5.79 (d, J = 6.1 Hz) and H-2 at δ_H 6.22 (d, J = 9.3 Hz), respectively, located two acetate moieties at C-9 and C-2. Since the ^{1}H and ^{13}C NMR spectra of compounds **3** and **1** were virtually identical, and similar NOEs were observed in both compounds, it was concluded that these compounds have the same stereochemistry at all of the ring junctures and chiral centers. Although brianthein U has not been reported, to maintain some coherence in the literature, compound **3** has been assigned the name 11-hydroxybrianthein U.

The (+)-APcIMS of compound **4** showed the pseudomolecular ion $[M + Na]^+$ at m/z 607/609 and significant fragments at m/z 479/481 $[M - H_2O - {}^nPrCOOH + H]^+$, 419/421 $[M - H_2O - AcOH - {}^nPrCOOH + H]^+$, and 359/361 $[M - H_2O - 2AcOH - {}^nPrCOOH + H]^+$ that were indicative of the molecular formula of $C_{28}H_{37}ClO_{11}$ which was confirmed by the HRFABMS of the ion at m/z 607.1925 (Δ −0.3 mmu). These data suggested that **4** is an isomer of **3**. Comparison of the ^{1}H and ^{13}C NMR data of **4** with those of **3** confirmed the isomeric structure of these compounds, differing only in the positions of the ester groups. Again, the location of the butyrate versus the acetate groups was deduced by gHMBC experiments. Thus, the butyrate group was located at C-2 position by the HMBC correlations between the butyrate carbonyl carbon resonance at δ_C 172.2 and H-2 at δ_H 6.27 (d, J = 9.6 Hz) and two methylene protons of the propyl group at δ_H 1.66 (m) and 2.30 (t, J = 7.4 Hz). The acetate groups were assigned to C-9 and C-12 by HMBC cross-peaks between the acetate carbonyl carbon resonances at δ_C 170.0 (s) and at δ_C 170.5 (s) and the H-9 at δ_H 5.78 (d, J = 6.5 Hz) and H-12 at δ_H 4.56 (d, J = 5.8 Hz), respectively. The relative stereochemistry of **4** was shown to be the same as in **3** by comparison of their NMR data and by a NOESY experiment of **4**. On the basis on these data, compound **4** was named 11-hydroxybrianthein Y.[12b]

Briarein **5** was isolated as a colorless solid. The pseudomolecular ion $[M + Na]^+$ observed in its (+)-APcIMS at m/z 637/639, along with the intense peaks at m/z 597/599 $[M - H_2O + H]^+$ and 509/511 $[M - H_2O -$

TABLE 2. ^{1}H NMR Spectral Data ($CDCl_3$) of Compounds 3−6

C no.	3	4	5	6
2	6.22 d, 9.3	6.27 d, 9.6	5.24 br s	5.26 br s
3	5.64 dd, 9.3, 11.5	5.63 dd, 9.6, 12.4	2.44 m, 2.61 m	2.41 m, 1.69 m
4	5.92 d, 11.5	5.92 d, 12.4	3.02 dt, 3.1, 13.9, 13.9, 2.06 m	3.01 br t, 13.2, 2.06 m
6	5.20 br s	5.19 br s	5.01 br s	5.03 br s
7	5.04 d, 4.1	5.04 d, 3.7	5.24 br s	5.26 br s
9	5.79 d, 6.1	5.78 d, 6.5	5.84 d, 4.4	5.87 d, 4.2
10	2.65 d, 6.1	2.68 d, 6.5	2.47 d, 4.4	2.47 d, 4.2
12	4.54 d, 5.7	4.56 d, 5.8	4.56 d, 5.9	4.55 d, 5.9
13	3.60 dd, 3.4, 5.7	3.58 dd, 3.5, 5.8	3.58 dd, 3.3, 5.8	3.57 dd, 3.6;5.9
14	2.94 d, 3.4	2.92 d, 3.5	2.92 d, 3.3	2.86 d, 3.6
15	1.19 s	1.19 s	1.18 s	1.22 s
16	6.37 br s, 5.92 br s	6.41 br s, 5.95 br s	5.90 br s, 5.84 br s	5.90 br s, 5.83 br s
17	2.41 q, 6.9	2.40 q, 7.2	2.40 m	2.42 m
19	1.24 d, 6.9	1.24 d, 7.2	1.20 d, 7.4	1.23 d, 7.4
20	1.30 s	1.31 s	1.30 s	1.30 s
OH-8	4.35 s	4.44 s	4.33 s	4.24 s
OH-11	3.80 s	3.90 s	3.47 s	3.34 s
ester at C-2[a]	2.06 s	2.30 t, 7.4	2.34 t, 7.4	2.06 s
		1.66 m	1.67 m	
		0.94 t 7.4	0.98 t 7.4	
ester at C-9	2.18 s	2.18 s	2.20 s	2.21 s
ester at C-12	2.35 t, 7.4	2.11 s	2.28 t, 7.4	2.35 t, 7.4
	1.70 m		1.63 m	1.70 m
	1.00 t, 7.4		0.93 t, 7.4	1.00 t, 7.4

[a] Ester = butyrate or acetate.

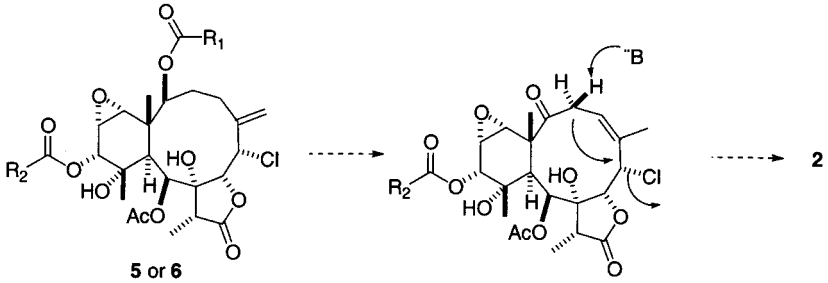
^{n}PrCOOH + H]$^+$ suggested the molecular formula for **5** of $C_{30}H_{43}ClO_{11}$, which was confirmed by HRFABMS of the [M + Na]$^+$ ion at *m/z* 637.2394 (Δ −0.2 umm). The difference of 2 mass units in the molecular formula of **5** in relation to that of **1** suggested that one of the double bonds in **1** must be hydrogenated in **5**. This was corroborated by the spectral data (^{1}H and ^{13}C NMR) of **5**, which were very similar to those of **1** but showed the presence of two additional methylene groups at δ_H 2.44, 2.61/δ_C 28.4, and δ_H 3.02, 2.06/32.9 in **5** instead of the olefinic signals corresponding to the Δ^3 double bound in **1** at δ_H 5.62/δ_C 130.0 at C-3 and δ_H 5.91/δ_C 128.1 at C-4, indicating that **5** must be the 3,4-dihydro derivative of **1**. gHMQC and ^{1}H−^{1}H COSY experiments of **5** allowed us to confirm this by an examination of the spin system from H-2 at δ_H 5.24 to H_2-4 at δ_H 2.06 (see Table 2). The acetate was located at C-9 as determined by the HMBC cross-peaks between the acetate carbonyl carbon resonance at δ_C 169.6 and the methyl protons of the acetate at δ_H 2.20 and H-9 at δ_H 5.84, while the other two butyrate moieties were assigned to C-2 and C-12 positions by HMBC correlations between H-2 to δ_C 172.9 and H-12 to δ_C 173.1.

The relative stereochemistry of **5** was determined by 2D NOESY and coupling constant analysis. Spectral comparison of **5** to compounds **1**−**4** showed that the stereochemistry of the substituents on the six-membered ring were identical in all the compounds. Key NOE correlations between H-2 at δ_H 5.24 and H-10 at δ_H 2.47 indicated that these protons are on the same face of the molecule (assigned as α) and the butyrate at C-2 must have a pseudoequatorial disposition. In addition, the NOE correlations between H-17 at δ_H 2.40 and the protons H-7 at δ_H 5.24 indicated that they are oriented on the same face of the molecule as in compounds **1**−**4**. On the basis on these data, compound **5** was named as 3,4-dihydro-11-hydroxybrianthein V.

HRFABMS established a molecular formula of $C_{28}H_{39}ClO_{11}$ for the last new briarein, compound **6**. This molecular formula requires the same degree of unsaturation but is 28 amu's lighter than **5**, suggesting than one of the butyrate groups in **5** was substituted by an acetate group in **6**. This was corroborated by the similarity of the ^{1}H and ^{13}C NMR spectral data of **6** to those of **5** but only differing in the presence of signals corresponding to an extra acetate group in compound **6** and the absence of the signals of one of the butyrate groups of compound **5**. Again, HMBC correlations between the butyrate carbonyl carbon resonance at δ_C 173.0 and the protons corresponding to H-12 at δ_H 4.55 and the butyrate methylene protons at δ_H 2.35 and 1.70, allowed us to locate the butyrate group at C-12. On the other hand, the HMBC cross-peaks between the acetate carbonyl carbon resonance at δ_C 169.5 and the acetyl methyl protons at δ_H 2.21 and H-9 at δ_H 5.87, and δ_C 170.3 and the acetyl methyl protons at δ_H 2.06, established the locations of the acetate groups at C-9 and C-2. The relative stereochemistry of **6** was confirmed in the same manner as **5** using coupling constant analysis and a NOESY experiment. Therefore, 3,4-dihydro-11-hydroxybrianthein U was assigned for compound **6**.

B. asbestitum is one of the most ubiquitous and chemically deterrent of the West Indian gorgonians.[5] This organism is somewhat unusual in providing such a number of structurally diverse diterpenes; the isolation of these six new briareins, including the unprecedented cyclobutenbriarein A, is another demonstration of the high capability of these organisms to biosynthesize new metabolites. The new diterpene cyclobutenbriarein A (**2**) contains a cyclobutene ring in a tricyclo[8.4.0.0^{3,6}]tetradec-4-ene ring system that has not previously been described either from natural or synthetic origins. The only example of a cyclobutene ring in a marine natural product is the cyclobutenone moiety of acetylcoriacenone and its isomer, both isolated from the brown alga *Pachydictyon*

SCHEME 1



coriaceum.[15] Although bicyclo[6.2.0]dec-8-ene has been subjected to numerous cycloaddition reactions,[16] this ring system has not been found in any natural product.

The diversity of novel diterpenes which have been isolated from *B. asbestinum* is unusual and raises the question of the origin of these metabolites. One possible explanation for this chemical diversity is that the microbial fauna/flora associated with this coral is responsible for terpene production and the chemical variation observed with coral extracts is due to differences in the microorganism population in the coral hosts.

The co-occurrence of cyclobutenbriarein A (**2**) with compounds **5** and **6** in the same organism suggests the biosynthetic pathway outlined in Scheme 1. An isomerization of the double bond from $\Delta^{5,16}$ to Δ^{4} and the oxidation of C-2 to a ketone functionality in compounds **5** or **6** would cause an increase of the acidity of protons at C-3, allowing the base-catalyzed removal of one those protons with a subsequent intramolecular nucleophilic substitution of the chorine at C-6 to yield cyclobutenbriarein A (**2**). The relative stereochemistry found in **2** agrees with a S_N2 mechanism for the last step of this biosynthetic pathway. This biosynthetic proposal could offer a biomimetic synthetic route to these novel bicyclo-[6.2.0]dec-8-ene ring systems.

Experimental Section

NMR spectra were recorded at 500/125 MHz (^{1}H/^{13}C) or 200/50 MHz (^{1}H/^{13}C) in $CDCl_3$. The manufacturer's software was used for DEPT, GATED, gradient-enhanced COSY, as well as for the inversed-detected gradient selected heteronuclear correlations gHMBC and gHMQC. In the long-range proton−carbon correlation experiment (GHMBC), a delay of 83 ms was used (calculated for $^nJ(CH) = 6$ Hz). NOESY experiments were performed using a mixing time of 800 ms. (+)-LRFABMS and (+)-HRFABMS were measured using thioglycerol with 1% of NaI as matrix. Optical rotations were determined using an Hg lamp at 590 nm. Semipreparative HPLC was performed using μ-Porasil columns (250 × 10 mm) with RI detection.

Collection, Extraction, and Isolation Procedures. *B. asbestinum* was collected using scuba (12 M) at Sweetings Cay in the Bahamas in 1999 and was frozen on collection and freeze-dried prior to extraction. A voucher specimen (UDC#9950) is held in the Dpto. de Química Fundamental, Universidade de A Coruña, A Coruña, Spain. The freeze-dried material (287 g) was extracted three times with methanol, and the combined methanol extracts were partitioned between CH_2Cl_2 and H_2O (1:1). The fraction soluble in CH_2Cl_2 was evaporated under reduced pressure and partitioned between 10% aqueous MeOH (250 mL) and hexane (3 × 250 mL). Water was added to the polar fraction to generate a 50% aqueous MeOH solution, and this was then extracted with CH_2Cl_2 (5 × 200 mL). After evaporation, the combined organic layers yielded 10.0 g of an oil from the hexane fraction and 9.5 g of an oil from the CH_2Cl_2 fraction. The product obtained from the methylene chloride fraction (9.5 g) was separated by flash column chromatography (silica gel 230−400 mesh, eluting with hexane/acetone mixtures of increasing polarity) to give several fractions. Purification of some portions of the individual fractions was achieved by normal-phase HPLC using acetone/hexane (2:8) to give compounds **1** (113 mg, 0.39% dry weight), **2** (6.2 mg, 0.53 × 10^{-2}% dry weight), **3** (19.8 mg, 2.0 × 10^{-2}% dry weight), **4** (21.9 mg, 3.6 × 10^{-2}% dry weight), **5** (37.8 mg, 3.2 × 10^{-2}% dry weight), and **6** (8.9 mg, 1.9 × 10^{-2}% dry weight). Percentage in relation to the total dry weight.

11-Hydroxybrianthein V (1): colorless crystals; $[\alpha]^{24}_D$ −124.4 (c = 1.155, $CHCl_3$); ^{1}H and ^{13}C NMR see Table 1; (+) LRFABMS (thioglycerol) m/z (rel intensity) 635/637 ([M + Na]$^+$; 11), 599 ([M − HCl + Na]$^+$; 10); (+) APcIMS m/z (rel intensity) 635/637 ([M + Na]$^+$; 91), 613/615 ([M + H]$^+$; 12), 595/597 ([M − H_2O + H]$^+$; 100), 525/527 ([M − ^{n}PrCOOH + H]$^+$; 38), 507/509 ([M − H_2O − ^{n}PrCOOH + H]$^+$; 53), 447/449 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 43), 419/421 ([M − H_2O − 2^{n}PrCOOH + H]$^+$; 60), 359/361 ([M − H_2O − AcOH − 2^{n}PrCOOH + H]$^+$; 62); HRFABMS m/z 635.2228 [M + Na]$^+$ (calcd for $C_{30}H_{41}ClO_{11}Na$, 635.2235).

Cyclobutenbriarein A (2): white solid; $[\alpha]^{27}_D$ −22.8 (c = 0.22, $CHCl_3$); IR ν_{max} (neat) 3406, 1778, 1739, 1705 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 222 (3.44), 264 (2.73) nm; ^{1}H and ^{13}C NMR see Table 1; (+) APcIMS m/z (rel intensity) 529 ([M + Na]$^+$; 6), 507 ([M + H]$^+$; 4), 489 ([M − H_2O + H]$^+$; 43), 341 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 20); (+) APcIMS (adding NaCl) m/z (rel intensity) 529 ([M + Na]$^+$; 82), 511 ([M − H_2O + Na]$^+$; 82), 489 ([M − H_2O + H]$^+$; 22), 341 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 31); (+) HRFABMS m/z 529.2046 [M + Na]$^+$ (calcd for $C_{26}H_{34}O_{10}Na$, 529.2050).

11-Hydroxybrianthein U (3): colorless solid; $[\alpha]^{28}_D$ −128.6 (c = 0.55, $CHCl_3$); ^{1}H and ^{13}C NMR see Tables 2 and 3; (+)-APcIMS m/z (rel intensity) 607/609 ([M + Na]$^+$; 43), 567/569 ([M − H_2O + H]$^+$; 100), 507/509 ([M − H_2O − AcOH + H]$^+$; 42), 419/421 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 62), 359/361 ([M − H_2O − 2AcOH − ^{n}PrCOOH + H]$^+$; 69); (+) HRFABMS m/z 607.1921 [M + Na]$^+$ (calcd for $C_{28}H_{37}ClO_{11}$-Na, 607.1922).

11-Hydroxybrianthein Y (4): white solid; $[\alpha]^{27}_D$ −103.3 (c = 0.37, $CHCl_3$); ^{1}H and ^{13}C NMR see Tables 2 and 3; (+) APcIMS m/z (rel intensity) 607/609 ([M + Na]$^+$; 66), 567/569 ([M − H_2O + H]$^+$; 100), 479/481 ([M − H_2O − ^{n}PrCOOH + H]$^+$; 41), 437/439 ([M − AcOH − ^{n}PrCOOH + H]$^+$; 27), 419/421 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 82), 359/361 ([M − H_2O − 2AcOH − ^{n}PrCOOH + H]$^+$; 42); (+) HRFABMS m/z 607.1925 [M + Na]$^+$ (calcd for $C_{28}H_{37}ClO_{11}Na$, 607.1922).

3,4-Dihydro-11-hydroxybrianthein V (5): white solid; $[\alpha]^{24}_D$ −61.3 (c = 1.085, $CHCl_3$); ^{1}H and ^{13}C NMR see Tables 2 and 3; (+) APcIMS m/z (rel intensity) 637/639 ([M + Na]$^+$; 50), 615/617 ([M + H]$^+$; 8), 597/599 ([M − H_2O + H]$^+$; 95), 527/529

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TABLE 3. ^{13}C NMR Spectral Data and DEPT-135 ($CDCl_3$) of Compounds 3−6

C no.	3	4	5	6
1	43.1 s	43.2 s	42.7 s	42.7 s
2	76.0 d	75.6 d	77.7 d	77.5 d
3	129.8 d	130.0 d	28.4 t	28.3 t
4	128.3 d	128.2 d	32.9 t	29.7 t
5	135.8 s	136.0 s	138.9 s	139.0 s
6	62.9 d	62.9 d	66.6 d	66.8 d
7	78.0 d	78.0 d	77.7 d	77.9 d
8	83.6 s	83.7 s	83.2 s	83.2 s
9	68.5 d	68.4 d	69.4 d	69.5 d
10	36.3 d	36.2 d	36.3 d	36.4 d
11	72.7 s	73.4 s	73.5 s	73.4 s
12	72.8 d	72.9 d	72.6 d	72.8 d
13	54.1 d	54.0 d	54.0 d	53.6 d
14	62.0 d	61.9 d	61.9 d	62.6 d
15	15.5 q	15.6 q	15.5 q	16.8 q
16	117.9 t	117.8 t	117.9 t	122.1 t
17	45.7 d	45.7 d	44.6 d	44.6 d
18	174.5 s	174.5 s	175.0 s	174.9 s
19	7.0 q	7.0 q	7.5 q	7.6 q
20	22.3 q	22.3 q	21.7 q	21.6 q
ester at C-2[a]	169.5 s	172.2 s	172.9 s	170.3 s
	21.0 q	36.3 t	35.8 t	21.0 t
		18.4 t	18.4 t	
		13.6 q	13.6 q	
ester at C-9	170.0 s	170.0 s	169.6 s	169.5 s
	21.9 q	21.9 q	21.9 q	21.9 q
ester at C-12	173.0 s	170.5 s	173.1 s	173.0 s
	36.2 t	20.4 q	36.3 t	35.8 t
	18.6 t		18.4 t	18.5 t
	13.7 q		13.6 q	13.6 q

[a] Ester = butyrate or acetate.

([M − ^{i}PrCOOH + H]$^+$; 45), 509/511 ([M − H_2O − ^{n}PrCOOH + H]$^+$; 100), 449/451 ([M − H_2O − AcOH − ^{n}PrCOOH + H]$^+$; 48), 421/423 ([M − H_2O − 2^{n}PrCOOH + H]$^+$; 67), 379/381 ([M − AcOH − ^{n}PrCOOH + H]$^+$; 83), 361/363 ([M − H_2O − AcOH − 2^{n}PrCOOH + H]$^+$; 82); (+) HRFABMS *m/z* 637.2394 [M + Na]$^+$ (calcd for $C_{30}H_{43}ClO_{11}Na$, 637.2392).

3,4-Dihydro-11-hydroxybrianthein U (6): white solid; $[\alpha]^{26}_D$ −50.8 (*c* = 0.185, $CHCl_3$); ^{1}H and ^{13}C NMR see Tables 2 and 3; (+) APcIMS *m/z* (rel intensity) 609/611 ([M + Na]$^+$; 32), 587/589 ([M + H]$^+$; 12), 569/571 ([M − H_2O + H]$^+$; 100), 527/529 ([M − AcOH + H]$^+$; 27), 509/511 ([M − H_2O − AcOH + H]$^+$; 60), 481/483 ([M − H_2O − ^{n}PrCOOH + H]$^+$; 33), 421/423 ([M − H_2O − 2AcOH + H]$^+$; 45), 379/381 ([M − 2AcOH − ^{n}PrCOOH + H]$^+$; 45), 361/363 ([M − H_2O − 2AcOH − ^{n}PrCOOH + H]$^+$; 41); (+) HRFABMS *m/z* 609.2074 [M + Na]$^+$ (calcd for $C_{28}H_{39}ClO_{11}Na$, 609.2079).

Crystallographic Data and X-ray Structure Analysis of 1. A small colorless plate of **1** was obtained by slow evaporation of a MeOH solution. The crystals belong to the trigonal space group *R*3 with *a* = 28.0784 Å, *b* = 28.0784 Å, *c* = 10.5712 Å, *U* = 7217.71 Å^3, *Z* = 9. Data were collected on a Nonius-Mach3 diffractometer using Ni-filtered Cu Kα (λ = 1.5418 Å) radiation. A total of 2299 reflections were used in the solution and refinement. The structure was solved by using WinGX software[17] (SIR-92 for solution and SHELXL-97 for refinement). For coordinates corresponding to the absolute stereochemistry represented (see the Supporting Information) a Flack parameter 0.004 ± 0.175 was obtained.

Acknowledgment. We are very grateful for HR-FABMS performed at the Centro de Instrumentación Científica of the University of Granada (Spain). This work was supported by grants from CICYT (MAR99-0287) and Xunta de Galicia (PGIDT99PXI10304A and PGIDT00MAR10301PN). N.G. thanks the Xunta de Galicia for a grant. R.G.K. acknowledges support provided by a Camille and Henry Dreyfus fellowship. Ship time aboard the R/V Bellows was generously provided by the Florida Institute of Oceanography.

Supporting Information Available: ^{1}H and ^{13}C, DEPT, and 2D NMR spectra for compounds **1**−**6**, X-ray data for compound **1**, and GATED and IR spectra for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0160221

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