Studies on the Minor Constituents of the Caribbean Gorgonian Octocoral *Briareum asbestinum* PALLAS. Isolation and Structure Determination of the Eunicellin-Based Diterpenoids Briarellins E—I

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Five new eunicellin-type diterpenoids, briarellins E—I, along with several known diterpenoids of the asbestinane, briarane and eunicellane classes, were isolated from the Caribbean gorgonian octocoral *Briareum asbestinum* collected in Puerto Rico. The structures of these compounds were established on the basis of spectroscopic evidence.

Key words eunicellin-based diterpenoid; cladiellin; *Briareum asbestinum*; Caribbean gorgonian octocoral; briarellin; cytotoxicity

Coelenterates of the orders Gorgonaceae and Alcyonaceae have been investigated extensively for many years and have yielded a plethora of novel secondary metabolites.[1] In both orders, the most commonly encountered class of compounds are derived from the 14-membered cembrane nucleus. In Caribbean waters, *Briareum asbestinum* is an exceedingly variable, encrusting soft coral which overgrows living and dead hard coral skeletons.[2] *Briareum* species of soft corals, which can be found on both the Caribbean and Pacific reefs, have produced a number of diterpenoid metabolites containing further cyclized cembrane ring systems. The first chemical investigations reported from *B. asbestinum* collected in the Caribbean led to the discovery of chlorinated compounds called the briareins.[3] Subsequent collections of *B. asbestinum* from Belize, Honduras, Puerto Rico, and Tobago have afforded over 30 structurally related derivatives known as the asbestinins.[4] Although produced by a different terpene cyclization pathway, both classes consist of bicyclic molecules with fused six- and ten-membered rings (see Chart 1).

In recent years, additional investigations have revealed that the genus *Briareum* also produces yet another class of metabolites called the eunicellins (cladiellins) which appear to be restricted to marine organisms.[5] Although many eunicellin-type diterpenoids have been isolated from Japanese, Australian, Micronesian, Chinese, Indian, South African, and Mediterranean soft corals,[6] briarellins A—D (**1**—**4**) are the only known eunicellin-type diterpenoids isolated to date from a Caribbean gorgonian species.[5b] These rare metabolites, isolated from Puerto Rican specimens of *B. asbestinum*, are the first diterpenoids of this class found to possess a seven-membered lactone ring formed between C-3 and C-16. That study also resulted in the isolation of 25 diterpenoids, of which 19 were asbestinin-type compounds,[4e,7] five had the eunicellin skeleton,[5b] and one had the briarein skeleton.[5b] The presence of all three classes of compounds provides circumstantial evidence for a biosynthetic pathway in which a cembrane skeleton is the precursor to the briarein skeleton (*via* C-3/C-8 cyclization), the eunicellin skeleton (*via* C-2/C-11 cyclization), and thus the asbestinin skeleton (Chart 1).[4a] In the present study we report the isolation and structural elucidation of five new members of the briarellin class, namely briarellins E—I (**5**—**9**), isolated from the same specimens of *B. asbestinum* collected at two different locations in Puerto Rico. Specimens collected near Palomino Island (east coast) yielded briarellins-G and -I and those collected near Mona Island (west coast) produced briarellins-E, -F, and -H. Briarellin-H (**8**) contains the same ε-lactone ring found in briarellins A—D (**1**—**4**) and the structures of briarellins -E (**5**), -F (**6**), -G (**7**), and -I (**9**) are distinguished by the presence of the same ether bridge across the C-3/C-16 positions found in asbestinin-type diterpenoids. Unlike briarellins A—D, the new eunicellin-type compounds reported here have the same relative configuration at C-11 found in the six-membered ring in nearly all the other metabolites of this class of marine metabolites.[6]

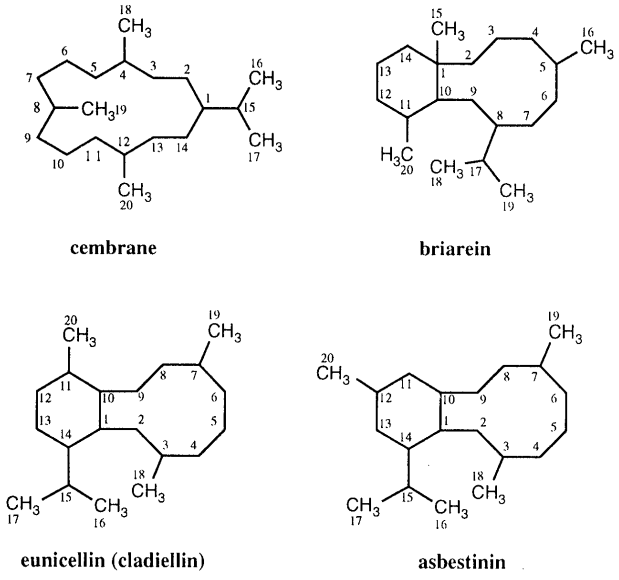

Chart 1

Extraction of the freeze-dried soft coral with a 1 : 1 mixture of chloroform–methanol followed by extensive chromatography on silica gel afforded five new compounds: briarellin-E (**5**), briarellin-F (**6**), briarellin-G (**7**), briarellin-H (**8**), and briarellin-I (**9**). Briarellin-E (**5**), the least polar compound (0.0010% dry weight), was obtained as a

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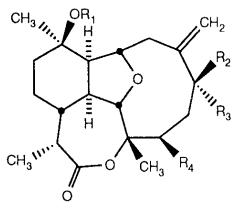
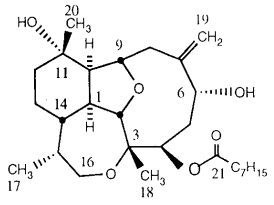


1 R_1=R_3=H; R_2=OH; R_4=$OCOC_7H_{15}$
2 R_1=R_2=H; R_3=OH; R_4=$OCOC_7H_{15}$
3 R_1=R_2=H; R_3=$OCOC_3H_7$; R_4= $OCOC_7H_{15}$
4 R_1=$OCOC_3H_7$; R_2=R_4=H; R_3=OH



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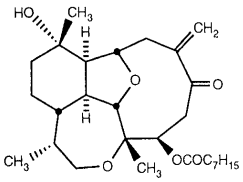

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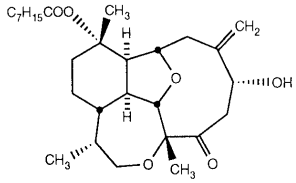

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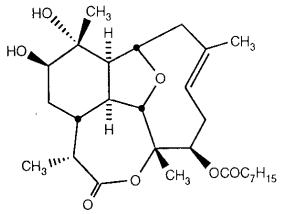

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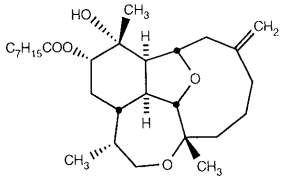

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colorless oil that analyzed for $C_{28}H_{46}O_6$. This result, obtained from a high-resolution (HR-)FAB-MS, indicated that **5** was a deoxy isomer of briarellin-B (**2**). The FAB-MS gave the highest mass peak at *m/z* 479 $[M+H]^+$ with fragments representing successive losses of one (*m/z* 461) molecule of H_2O, one (*m/z* 317) of caprylic acid, and another (*m/z* 299) molecule of H_2O. Its infrared (IR) spectrum indicated the presence of only one ester functionality (*vs.* two in **2**) at 1737 cm^{-1}, and contained a broad absorption for hydroxyl groups near 3425 cm^{-1}. Subtraction of the 8 carbons associated with the aliphatic ester group left 20 carbons, suggestive of a diterpene skeleton (5 unsaturations). Since the ^{13}C-NMR spectrum (Table 2) contained only two olefinic resonances [δ 148.22 (s), 115.14 (t) assigned to an exocyclic olefin] and one carbon resonance at δ 175.07 (s) for an ester carbonyl, the molecule was judged to be tetracyclic. The ^{1}H-NMR spectrum of briarellin-E (**5**) (Table 1) exhibited resonances due to a β-capryloxy proton [δ 5.17 (1H, br s)], an exocyclic methylene [δ 5.47 (1H, br s), 5.17 (1H, br s)], two oxymethine protons [δ 4.50 (1H, br t, J=3.3 Hz), 3.82 (1H, d, J=9.3 Hz)], two diastereotopic oxymethylene protons [δ 3.60 (1H, d, J=12.9 Hz), 3.38 (1H, dd, J=2.4, 13.2 Hz)], and an α-hydroxyl [δ 4.18 (1H, br s)] proton along with four methyl groups [δ 1.32 (3H, s), 1.31 (3H, s), 0.87 (3H, t, J=6.0 Hz), 0.81 (3H, d, J=6.9 Hz)]. The ^{1}H-NMR spectrum of **5** was similar to that which has been reported for briarellin-B (**2**) (see Table 1).[5b] The only major differences are the presence in **5** of two diastereotopic oxymethylene protons near δ 3.60 and 3.38 (ascribable to the C-16 protons) and the highfield shift experienced by Me-17, which resonates at δ 0.81 in **5** but appears at δ 1.36 in **2**.[5b] Comparison of the ^{13}C-NMR spectrum of briarellin-E (**5**) with that of briarellin-B (**2**) (see Table 2) confirmed the structural similarity of these two compounds and revealed the presence of some features unique to **5**. While the complex tetracyclic ring system along with the caprylate, hydroxyl, and exomethylene functionalities at C-4, C-6, and C-7, respectively, were shown to be intact in **5** on the basis of similar IR, NMR (^{1}H- and ^{13}C-), MS, and UV data, briarellin-E, however, did not show a lactone carbonyl signal near δ 175.92 ascribable in **2** to C-16. Instead, briarellin-E contained a new signal at δ 67.39 (t) which showed $^1J_{CH}$ correlations with its corresponding ^{1}H-NMR resonances at δ 3.60 and 3.38 during a heteronuclear chemical shift correlation with broad-band decoupling (CSCMBB) ($J \cong 140$ Hz) experiment. That carbon C-16 had indeed undergone complete reduction to form the same oxepane ring system present in the asbestinane diterpenoids was evident by NMR from the highfield shift experienced by atoms near that position. For instance, C-15, which resonates at δ 45.63 in **2**, appears at δ 36.43 in **5** whereas H-15 resonates at δ 1.58 (1H, m) *vs.* δ 2.92 (1H, m) in **2**. Likewise, highfield shifts were experienced by the carbon and hydrogen atoms at position 17.

Except for C-11, much of the stereochemistry of briarellin-E (**5**), which was fully defined by NMR (^{1}H- and ^{13}C-) and a phase-sensitive nuclear Overhauser effect spectroscopy (NOESY) experiment (Table 3), was found to be similar to that previously reported for briarellin-B (**2**).[5b] As noted before, the C-11 stereochemistry in briarellin-E did not compare favorably with that found in **2**. The cyclohexane structural unit in **5**, like in all the asbestinins, is locked in a boat conformation. The Me-20 substituent, which resonates at δ 1.31 and is located in a flagpole (axial) position, exhibited strong NOE responses with H-9 and H-14 both in the β-orientation. The determination of the β-orientation of Me-20 was also consistent with the proposed stereochemistry on the basis that it failed to exhibit an NOE response correlating it with H-10. The change in stereochemistry at position 11 in **5** is based also on the chemical shift difference of the C-11 carbon (δ 71.63) which appears shifted downfield to 81.41 ppm in **2**. A similar empirical relationship between the C-11 carbon chemical shift with the α and β configuration of the methyl substituent (Me-20) was observed for other compounds in this series throughout this work.[5b] Unlike in briarellins A—D (**1**—**4**), the relative stereochemistry at C-11 in briarellin-E (**5**) compares favorably with that found in most of the eunicellin-based compounds isolated from Pacific soft corals.[6]

The HR-EI-MS established a molecular formula for briarellin-F (**6**) of $C_{28}H_{44}O_6$. Thus, compound **6** possessed a mass spectral molecular ion which was only 2 amu's smaller than that of briarellin-E (**5**). Ions corresponding to sequential losses of H_2O (*m/z* 458) and of caprylic acid (*m/z* 314) from the molecular ion *m/z* 476 were evident in the HR-EI-MS. Compound **6** showed IR absorptions (3459, 1733, 1689 cm^{-1}) that indicated the presence of a

Table 1. ^{1}H-NMR (300 MHz) Spectral Data of the Briarellins E—I in $CDCl_3$

H	Briarellin-E (**5**) δ, mult, J (Hz), intgr	Briarellin-F (**6**) δ, mult, J (Hz), intgr	Briarellin-G (**7**) δ, mult, J (Hz), intgr	Briarellin-H (**8**) δ, mult, J (Hz), intgr	Briarellin-I (**9**) δ, mult, J (Hz), intgr	Briarellin-B (**2**) δ, mult, J (Hz), intgr
H_1	2.59, m, 1H	2.66, m, 1H	2.92, m, 1H	2.28, m, 1H	2.52, m, 1H	2.81, m, 1H
H_2	3.82, d, 9.3, 1H	3.81, d, 9.3, 1H	3.81, d, 9.0, 1H	3.89, d, 10.8, 1H	3.73, d, 9.6, 1H	3.83, d, 4.8, 1H
H_4	5.17, bs, 1H	5.77, dd, 5.4, 9.6, 1H	—	5.01, dd, 7.8, 9.9, 1H	2.82, m, 1H	4.64, t, 4.2, 1H
$H_{4'}$	—	—	—	—	2.49, m, 1H	—
H_5	1.75, m, 1H	2.80, d, 5.1, 1H	2.67, bs, 1H	1.97, m, 1H	1.67, m, 1H	2.76, t, 4.2, 1H
$H_{5'}$	1.75, m, 1H	2.80, d, 5.1, 1H	2.03, bs, 1H	2.96, m, 1H	1.30, m, 1H	1.76, m, 1H
H_6	—	—	—	5.43, t, 8.4, 1H	2.28, m, 1H	4.18, br m, 1H
$H_{6'}$	4.18, bs, 1H	—	4.46, bs, 1H	—	2.28, m, 1H	—
H_8	2.35, d, 2.7, 1H	2.38, dd, 3.0, 13.2, 1H	2.27, bd, 2.4, 1H	2.51, d, 6.9, 1H	2.18, m, 1H	2.49, dd, 3.3, 14.4, 1H
$H_{8'}$	2.35, d, 2.7, 1H	3.32, dd, 6.6, 13.2, 1H	2.73, d, 15.0, 1H	1.88, d, 13.8, 1H	3.38, dd, 6.9, 13.5, 1H	2.28, dd, 3.3, 15.6, 1H
H_9	4.50, t, 3.3, 1H	4.54, dt, 3.3, 3.6, 1H	4.46, m, 1H	4.48, d, 6.3, 1H	4.50, bt, 5.4, 1H	4.22, dd, 3.0, 6.3, 1H
H_{10}	2.27, m, 1H	2.16, bdd, 3.9, 11.7, 1H	3.22, bd, 10.2, 1H	2.01, bd, 7.5, 1H	2.23, m, 1H	3.05, dd, 6.3, 10.8, 1H
H_{12}	1.92, m, 1H	1.85, m, 1H	2.17, m, 1H	4.31, d, 4.2, 1H	—	1.99, m, 1H
$H_{12'}$	1.81, m, 1H	1.85, m, 1H	2.17, m, 1H	—	4.87, dd, 6.3, 8.1, 1H	1.99, m, 1H
H_{13}	1.48, m, 1H	1.57, m, 1H	1.82, m, 1H	2.13, m, 1H	1.47, m, 1H	1.81, m, 1H
$H_{13'}$	1.48, m, 1H	1.57, m, 1H	1.82, m, 1H	2.06, m, 1H	2.12, m, 1H	1.81, m, 1H
H_{14}	1.58, m, 1H	1.57, m, 1H	1.95, m, 1H	2.29, m, 1H	1.72, m, 1H	1.73, m, 1H
H_{15}	1.58, m, 1H	1.58, m, 1H	1.67, m, 1H	2.56, bd, 6.9, 1H	1.58, m, 1H	2.92, m, 1H
H_{16}	3.38, dd, 2.4, 13.2, 1H	3.40, dd, 3.3, 12.9, 1H	3.51, dd, 3.6, 13.2, 1H	—	3.48, dd, 2.1, 13.2, 1H	—
$H_{16'}$	3.60, d, 12.9, 1H	3.62, d, 13.2, 1H	3.67, d, 13.2, 1H	—	3.67, d, 13.2, 1H	—
Me_{17}	0.81, d, 6.9, 3H	0.84, d, 6.9, 3H	0.99, d, 6.7, 3H	1.37, d, 7.5, 3H	0.97, d, 10.8, 1H	1.36, d, 6.9, 3H
Me_{18}	1.32, s, 3H	1.30, s, 3H	1.30, s, 3H	1.46, s, 3H	1.28, s, 3H	1.37, s, 3H
H_{19}	5.17, s, 1H	5.35, s, 1H	5.12, s, 1H	1.81, s, 3H	5.30, s, 1H	5.73, br s, 1H
$H_{19'}$	5.47, s, 1H	5.43, bs, 1H	5.30, s, 1H	—	5.22, s, 1H	5.13, br s, 1H
Me_{20}	1.31, s, 3H	1.33, s, 3H	1.42, s, 3H	1.25, s, 3H	1.36, s, 3H	1.33, s, 3H
H_{22}	2.31, dd, 2.4, 6.9, 2H	2.31, t, 7.2, 2H	2.30, t, 7.2, 2H	2.35, t, 7.2, 2H	2.38, t, 7.8, 2H	2.36, t, 7.5, 2H
H_{23}	1.63, m, 2H	1.61, m, 2H	1.63, m, 2H	1.62, m, 2H	1.63, m, 2H	1.64, m, 2H
H_{24}	1.26, m, 2H	1.27, m, 2H	1.25, m, 2H	1.24, m, 2H	1.25, m, 2H	1.29, m, 2H
H_{25}	1.26, m, 2H	1.27, m, 2H	1.25, m, 2H	1.24, m, 2H	1.25, m, 2H	1.29, m, 2H
H_{26}	1.26, m, 2H	1.27, m, 2H	1.25, m, 2H	1.24, m, 2H	1.25, m, 2H	1.29, m, 2H
H_{27}	1.26, m, 2H	1.27, m, 2H	1.25, m, 2H	1.24, m, 2H	1.25, m, 2H	1.29, m, 2H
H_{28}	0.87, t, 6.0, 3H	0.87, t, 5.4, 3H	0.86, t, 6.9, 3H	0.87, t, 6.9, 3H	0.88, bt, 4.8, 3H	0.87, br t, 6.9, 3H

Assignments were aided by ^{1}H–^{1}H COSY, spin splitting patterns, selective decoupling experiments and comparison of J values (Hz). The δ values are in ppm and are referenced to the residual $CHCl_3$ signal (7.26 ppm).

Table 2. ^{13}C-NMR Spectral Data (75 MHz; $CDCl_3$)[a] of the Briarellins E—I

C	Briarellin-B (**2**) δ (mult)	Briarellin-E (**5**) δ (mult)	Briarellin-F (**6**) δ (mult)	Briarellin-G (**7**) δ (mult)	Briarellin-H (**8**) δ (mult)	Briarellin-I (**9**) δ (mult)
1	44.65 (d)	39.43 (d)	40.05 (d)	39.19 (d)	39.43 (d)	40.11 (d)
2	92.12 (d)	92.20 (d)	92.05 (d)	93.72 (d)	87.13 (d)	92.74 (d)
3	73.90 (s)	76.70 (s)	77.21 (s)	77.57 (s)	76.88 (s)	77.21 (s)
4	72.74 (d)	71.63 (d)	72.05 (d)	208.58 (s)	73.79 (d)	37.66 (t)
5	38.23 (t)	37.06 (t)	45.69 (t)	47.33 (t)	28.59 (t)	29.68 (t)
6	72.03 (d)	74.03 (d)	200.74 (s)	78.88 (d)	123.53 (d)	36.04 (t)
7	145.42 (s)	148.22 (s)	146.39 (s)	146.49 (s)	131.55 (s)	146.65 (s)
8	39.77 (t)	38.98 (t)	42.67 (t)	42.22 (t)	43.97 (t)	42.64 (t)
9	82.87 (d)	81.96 (d)	79.99 (d)	81.01 (d)	78.70 (d)	79.61 (d)
10	47.38 (d)	51.85 (d)	53.83 (d)	46.42 (d)	50.59 (d)	49.38 (d)
11	81.41 (s)	71.63 (s)	71.13 (s)	81.09 (s)	72.17 (s)	71.92 (s)
12	29.93 (t)	39.60 (t)	38.56 (t)	31.70 (t)	83.55 (d)	76.26 (d)
13	16.31 (t)	24.83 (t)	24.06 (t)	18.52 (t)	19.68 (t)	32.68 (t)
14	36.85 (d)	38.85 (d)	38.56 (d)	39.03 (d)	31.15 (d)	37.03 (d)
15	45.63 (d)	36.43 (d)	36.33 (d)	35.60 (d)	43.94 (d)	35.94 (d)
16	175.92 (s)	67.39 (t)	67.63 (t)	67.63 (t)	174.25 (s)	68.03 (t)
17	17.88 (q)	10.37 (q)	10.65 (q)	10.33 (q)	19.43 (q)	10.66 (q)
18	22.58 (q)	17.89 (q)	18.42 (q)	21.52 (q)	22.41 (q)	24.17 (q)
19	118.43 (t)	115.14 (t)	116.10 (t)	116.86 (t)	19.15 (q)	114.60 (t)
20	28.39 (q)	28.73 (q)	28.60 (q)	29.22 (q)	21.76 (q)	25.70 (q)
21	174.99 (s)	175.07 (s)	173.18 (s)	172.89 (s)	173.15 (s)	173.84 (s)
22	34.60 (t)	34.78 (t)	34.47 (t)	34.10 (t)	34.59 (t)	34.51 (t)
23	24.86 (t)	25.10 (t)	25.12 (t)	24.93 (t)	24.98 (t)	25.00 (t)
24	28.99 (t)[b]	28.94 (t)[b]	28.98 (t)[b]	28.89 (t)[b]	29.02 (t)[b]	29.06 (t)[b]
25	28.86 (t)[b]	28.85 (t)[b]	28.98 (t)[b]	28.99 (t)[b]	28.87 (t)[b]	28.94 (t)[b]
26	31.57 (t)	31.68 (t)	31.70 (t)	31.60 (t)	31.61 (t)	31.65 (t)
27	22.55 (t)	22.59 (t)	22.63 (t)	22.57 (t)	22.55 (t)	22.59 (t)
28	14.02 (q)	13.99 (q)	14.07 (q)	14.04 (q)	14.03 (q)	14.05 (q)

a) Multiplicities were obtained by an attached proton test (APT) experiment. Assignments were made on the basis of heteronuclear chemical shift correlation methods, carbon atom multiplicities and chemical shift values. The δ values are in parts per million and are referenced to the $CDCl_3$ signal (77.0 ppm). *b*) Values with identical superscripts in each column may be interchanged.

Table 3. Selected NOE Correlations of Briarellins E—I[a]

Atom	Briarellin-E	Briarellin-F	Briarellin-G	Briarellin-H	Briarellin-I
1	H_{17}	H_4, H_{10}, H_{17}	H_{10}, H_{17}	H_4	H_{10}
2	H_{14}, H_{18}	H_{15}, H_{18}	H_{14}, H_{18}, H_{20}	H_{20}	H_{14}, H_{18}, H_{20}
4	H_{10}	H_1	—	H_1	—
6	H_{18}	—	H_{18}	—	—
9	H_{20}	H_{20}	—	H_{20}	H_{20}
10	—	H_1	H_1	H_{12}	H_1
12	—	—	—	H_{10}	—
12′	—	—	—	—	H_{20}
13	H_{17}	—	H_{17}	—	H_{17}
14	H_2, H_{20}	H_{20}	H_2, H_9, H_{20}	—	H_2, H_{20}
15	—	H_2, $H_{16'}$	$H_{16'}$	—	$H_{16'}$
16′	—	H_{15}	H_{15}	—	H_{15}
Me_{17}	H_1, H_{13}	H_1	H_1, H_{13}	—	H_{13}
Me_{18}	H_2, H_6	H_2	H_2, H_6	—	H_2
Me_{20}	H_9, H_{14}	H_9, H_{14}	H_2, H_{14}	H_2, H_9	H_2, H_9, $H_{12'}$, H_{14}

a) Spectra were recorded at room temperature in $CDCl_3$ solutions.

hydroxyl, an ester, and an α,β-unsaturated carbonyl group, respectively. The ^{13}C-NMR signals at δ 200.74 (s), 146.39 (s), and 116.10 (t), together with the fact that **6** was UV-active (λ_{max} 224 nm), indicated that the carbonyl was conjugated to an exocyclic methylene group. The presence of the methylene proton signals at δ 5.43 (1H, s) and 5.35 (1H, s) in the ^{1}H-NMR spectrum confirmed this assignment. In general, comparisons between the ^{1}H- and ^{13}C-NMR spectra of **5** and **6** (see Tables 1, 2) confirmed the great similarity between both structures. The differences observed clearly suggested that these compounds differed only in their oxidation level at C-6.

Compound **7**, named briarellin-G, was isolated as a colorless oil with a molecular formula of $C_{28}H_{44}O_6$, estimated from ^{1}H- and ^{13}C-NMR data and confirmed by FAB mass spectrometry. These results indicated that **7** was an isomer of briarellin-F (**6**). The presence of fragment ions in the HR-EI-MS corresponding to $M^+ - 144$ and $M^+ - 144 - 18$ confirmed the presence in **7** of the caprylate and alcohol groups. The IR spectrum contained absorptions of hydroxyl (3453 cm^{-1}), ester (1733 cm^{-1}) and ketone (1701 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 1) contained signals for two exocyclic methylene protons at δ 5.30 (1H, s) and 5.12 (1H, s) and a broad signal at δ 4.46 (br s) assigned to an allylic α-hydroxyl proton (the latter correlated in the ^{13}C-NMR spectrum to a carbon resonance at δ 78.88). The absence of a 1H signal in the ^{1}H-NMR spectrum that could be ascribed to a β-capryloxy proton combined with the fact that the resonance line ascribed to C-11 appears in **7** somewhat shifted downfield (Table 2), clearly indicated the locus of the caprylate ester group at C-11. The ^{13}C-NMR signal at δ 208.58 (s) and the fact that briarellin-G was UV-inactive, indicated that the carbonyl was not in conjugation with the exocyclic methylene group. Therefore, the ketone functionality in **7** must be placed at C-4. The placement of the latter functionality at C-4 was consistent with the downfield shift experienced by atoms (^{1}H and ^{13}C) near that position (*i.e.* Me-18, C-5) (see Tables 1, 2). The chemical shift value observed for C-4 in **7** is in full agreement with the chemical shift values assigned to the carbonyl group in the known 4-oxoasbestinin series.[4d,e] The ^{13}C-NMR data together with the measured coupling constants and a NOESY experiment allowed us to determine unambiguously the relative stereochemistry of the various chiral centers in briarellin-G.

Briarellin-H (**8**), also obtained as a colorless oil, corresponded to a molecular formula of $C_{28}H_{44}O_7$ on the basis of its ^{13}C-NMR and HR-EI-MS ([M^+] *m/z* 492.31037) and contained seven unsaturations. The IR spectrum contained two ester bands at 1733 and 1718 cm^{-1} and a strong hydroxyl stretching absorption at 3472 cm^{-1}. The ^{1}H-NMR spectrum contained signals for an endocyclic sp^2 methine [δ 5.43 (1H, t, J=8.4 Hz)], a β-capryloxy proton [δ 5.01 (1H, dd, J=7.8, 9.9 Hz)], three oxymethine protons [δ 4.48 (1H, d, J=6.3 Hz), 4.31 (1H, d, J=4.2 Hz), 3.89 (1H, d, J=10.8 Hz)] along with five methyls [δ 1.81 (3H, s), 1.46 (3H, s), 1.37 (3H, d, J=7.5 Hz), 1.25 (3H, s), 0.87 (3H, t, J=6.9 Hz)]. The absence in the ^{1}H-NMR spectrum of two diastereotopic oxymethylene protons combined with the presence of a ^{13}C-NMR signal at δ 174.25 (s) indicated the presence in briarellin-H (**8**) of an ε-lactone functionality similar to that found in briarellins A—D (**1**—**4**). The ^{13}C-NMR signals at δ 131.55 (s) and 123.53 (d), together with the presence in the ^{1}H-NMR spectrum of a vinyl methyl signal at δ 1.81 (3H, s), suggested that the double bond in **8** had shifted to an endocyclic position. The *E* stereochemistry of the double bond was established on the basis of the highfield ^{13}C-NMR chemical shift value of the Me-19 (δ 19.15) resonance line.[4c] The relative locus of the two hydroxyl groups in briarellin-H was determined unambiguously through a selective insensitive nuclei enhanced by polarization transfer (INEPT) experiment. Selective irradiation of the signal at δ 4.31 (1H, d, J=4.2 Hz), ascribed to H-12, caused enhancement of the ^{13}C-NMR signals at δ 72.17 (C-11) and 21.76 (C-20). This experiment plus a ^{1}H–^{1}H correlation spectroscopy (COSY) experiment allowed us to place unambiguously the second hydroxyl group in **8** at C-12. The *trans* relative stereochemistry shown for the two hydroxyl groups at C-11 and C-12 was based on a strong NOE response observed between H-12 (δ 4.31) and H-10 (δ 2.01). Moreover, the lack of NOE between H-12 and Me-20 (δ 1.25) combined with the strong

upfield shift experienced by the Me-20 resonance (δ 21.76) in the ^{13}C-NMR spectrum argued for a *trans* 11,12-diol arrangement. Since the C-11, 12 constellation has been correlated with the C-10 array through their NOE's, the relative configuration of these chiral centers, therefore, must be as shown in structure **8**. The relative stereochemistry for all the remaining substituents on the complex tetracyclic array was determined to be identical to those of known briarellins A—D (**1**—**4**) by analysis of proton–proton coupling constants (see Table 1), NOE experiments (Table 3), and ^{1}H- and ^{13}C-NMR chemical shift comparisons (see Tables 1, 2). Curiously, of all the new compounds reported here, briarellin-H (**8**) was the only one prone to decomposition upon prolonged storage, even at $-10\,^{\circ}$C.

A molecular formula of $C_{28}H_{46}O_5$ was established for briarellin-I (**9**) from HR-EI-MS (462.33296, Calcd 462.33452), plus ^{1}H- and ^{13}C-NMR data (see Tables 1, 2). Fragment ions in the HR-MS corresponding to $M^+ - 144$ $[M-C_8H_{16}O_2]^+$ and $M^+ - 162$ $[M-C_8H_{16}O_2 - H_2O]^+$, confirmed the presence in **9** of one alcohol and one caprylate group. Subtraction of the 8 carbons, 16 hydrogens and 2 oxygens associated with the aliphatic ester group left a $C_{20}H_{30}O_3$ fragment suggestive of a 4-deoxy eunicellin-based diterpenoid skeleton. Absorptions in the IR indicated the presence of alcohol (3466 cm^{-1}) and ester carbonyl (1734 cm^{-1}) functionalities. A sharp signal at δ 4.87 (1H, dd, J=6.3, 8.1 Hz), assigned to an α-capryloxy proton, was correlated in the ^{13}C-NMR spectrum to a carbon resonance at δ 76.26 (d). Placement of the caprylate at C-12 was confirmed from the long range coupling observed between the oxymethine proton signal at δ 4.87 (H-12) and the ^{13}C signals at δ 173.84 (s, C-21), 71.92 (s, C-11), 49.38 (d, C-10), and 25.70 (q, C-20). The relative stereochemistry of the upper left hand quadrant of briarellin-I, which was fully defined by a phase-sensitive NOESY experiment (Table 3), was found to be opposite at C-12 to that previously established for briarellin-H (**8**). A large NOE crosspeak was observed for the methine proton at C-12 (δ 4.87) with the Me-20 (δ 1.36) group located at C-11 suggesting that these protons are on the same side of the cyclohexane ring assigned arbitrarily as the β-face. The determination of the β-orientation of H-12 in the cyclohexane ring was also consistent with the proposed stereochemistry on the basis of its lack of NOE response with H-10 [a strong NOE correlation between H-12 and H-10, both in the α-orientation, was observed in the NOESY spectrum of briarellin-H (**8**)]. The Me-20 group, located in a flagpole (axial) position, exhibited strong NOE responses with H-14 and H-9 thus establishing the orientation of the latter resonances in the β-orientation. From extensive ^{1}H–^{1}H COSY experiments it was possible to determine two separate spin systems within the complex tetracyclic ring moiety which map out the proton sequences H-4 to H-6 as well as the remaining protons around the cyclohexane, tetrahydrofuran, oxepane, and 10-membered carbocyclic rings including the more remote exocyclic methylene protons. From these experiments, plus the NMR (^{1}H- and ^{13}C-) and HR-EI-MS, it was established conclusively that briarellin-I (**9**) lacked an alkanoyloxy group at C-4. Thus, compound **9**, with these unique structural features, represents the second 4-deoxy eunicellin-based diterpenoid isolated from a Caribbean specimen of *B. asbestinum*.[5b]

The carbon skeleton of the asbestinins is related to that of the eunicellins and cladiellins by migration of a methyl group from C-11 to C-12 (Chart 1). Since it has been noted that briarellins A—I co-occur within the same specimen of *B. asbestinum* along with many asbestinin and briarein-type diterpenes, an important finding for purposes of this study would be the consistency of these observations with a biosynthetic path wherein a cembrane intermediate serves as a precursor to the briarein, eunicellin and asbestinin skeletons. As has been noted here briarellins A—D (**1**—**4**) have opposite relative configuration at C-11 from that found in briarellins E—I (**5**—**9**). The biosynthetic hypothesis that asbestinins are derived from briarellins[5b] *via* a 1,2-methyl shift is in accord with the fact that the methyl groups in briarellins E—I (**5**—**9**) are on the same sides of the ring consistent with a suprafacial migration. On the other hand, the occurrence of briarellins A—D (**1**—**4**) within the same organism appears to negate this hypothesis since the methyl groups, which are on opposite sides of the ring, would require an impossible antarafacial migration.

Experimental

General Experimental Procedures IR spectra were recorded on a Nicolet 600 FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a General Electric Multinuclear QE-300; ^{1}H-NMR chemical shifts are recorded with respect to the residual $CHCl_3$ signal (7.26 ppm) and ^{13}C-NMR chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm). Optical rotations were determined on a Perkin–Elmer Polarimeter Model 243B. HR-EI and both high- and low-resolution (HR-, LR-) FAB$^+$ MS were determined in the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln. Column chromatography was performed on Analtech Si gel (35—75 mesh) and TLC analyses were carried out using Analtech glass packed precoated Si gel plates. All solvents used were either spectral grade or were distilled from glass prior to use.

Collection, Extraction, and Isolation Minced and freeze-dried specimens of *B. asbestinum* (4.01 kg) collected at Mona Island, Puerto Rico were extracted exhaustively with $CHCl_3$–MeOH (1 : 1) (7 × 1 l) and after filtration the crude extract was evaporated under vacuum to yield a residue (300.7 g) that was partitioned against H_2O with hexane and $CHCl_3$ (6 × 1 l). The hexane extract was subsequently filtered and the filtrate was concentrated *in vacuo* to yield 208.4 g of a dark green oily residue. The toluene soluble portion (156.9 g) was fractionated by size exclusion chromatography on a Bio-Beads SX-2 column. The combined diterpene-containing fractions (TLC guided) were concentrated to an orange oil (41.4 g) and chromatographed over a Si gel column (800 g) with 10% EtOAc in hexane. The less polar portion of the lipids was fractionated roughly into fractions A through J on the basis of TLC analyses. Subsequent purification of fractions A—I led to the isolation of 19 known diterpenoids of the asbestinane class.[4e,7] Fraction J (*ca.* 11.3 g) was fractionated over a Si gel column (350 g) with 1% MeOH in $CHCl_3$ into 14 subfractions (1—14). Subfractions 1—13 were extensively chromatographed to give the known briarellins A—D (**1**—**4**), *seco*-briarellin, and briarein-A.[5b] Subfraction 13 (*ca.* 867.5 mg) was fractionated further *via* HPLC [ODS Si gel with 20% H_2O in MeOH] to yield pure briarellin-H (**8**) (18.0 mg, 0.0004% dry wt.). Briarellin-E (**5**) (41.0 mg, 0.0010% dry wt.) and briarellin-F (**6**) (3.7 mg, 0.00009% dry wt.) were isolated from subfraction 14 (*ca.* 696.7 mg) *via* reversed-phase HPLC using an Ultrasphere ODS Si gel column [MeOH–H_2O (85 : 15)]. Specimens of *B. asbestinum* collected near Palomino Island, Puerto Rico (*ca.* 1.6 kg, dry wt.) were fractionated as described previously in a recent report.[4e] Subfractions 9 and 39 obtained from the hexane solubles (*ca.* 52.5 g) yielded briarellins-G (**7**) and -I (**9**), respectively. Subfraction 9 (*ca.* 1.0 g) was extensively fractionated *via* repeated Si gel

column chromatography using mixtures of EtOAc in hexane (10% to 30%) and HPLC (Partisil 5 Si gel in 45% EtOAc in hexane) to give 13.2 mg (0.0003% dry wt.) of briarellin-G (**7**). Briarellin-I (**9**) was isolated from subfraction 39 (*ca.* 492 mg) *via* column chromatography on Si gel (10% isopropanol in hexane) yielding 6.9 mg of pure compound (0.0002% dry wt.).

Briarellin-E (**5**): Colorless oil: $[\alpha]_D^{28}$ −25.24° (c=14.9, $CHCl_3$). IR (neat): 3425, 3078, 1737 cm^{-1}. FAB-MS m/z (%): 479 $[M+H]^+$ (6), 461 (75), 317 (33), 299 (35), 219 (23), 136 (72), 105 (80), 91 (100). HR-FAB-MS, Calcd for $C_{28}H_{46}O_6Na$ $[M+Na]^+$: 501.31921. Found: 501.320200. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Briarellin-F (**6**): Colorless oil: $[\alpha]_D^{30}$ −30.79° (c=1.27, $CDCl_3$). IR (neat): 3459, 3076, 1733, 1689 cm^{-1}. UV λ_{max}^{MeOH}: 224 ($\log\varepsilon$ 3.44), EI-MS m/z (%): 476 (M^+, 3.7), 458 (9.4), 332 (5.6), 314 (7.8), 274 (65.8), 219 (17.8), 193 (35.7), 175 (20.0), 122 (82.6), 57 (100). HR-EI-MS, Calcd for $C_{28}H_{44}O_6$ (M^+): 476.31378. Found: 476.31242. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Briarellin-G (**7**): Colorless oil: $[\alpha]_D^{30}$ +33.24° (c=2.49, $CHCl_3$). IR (neat): 3453, 3070, 1733, 1701 cm^{-1}. FAB-MS m/z: 499 $[M+Na]^+$. EI-MS m/z (%): 332 ($M^+-C_8H_{16}O_2$, 11.6), 318 (25.7), 314 (1.6), 300 (1.3), 208 (41.2), 148 (17.8), 111 (23.9), 69 (48), 57 (78.6). HR-EI-MS, Calcd for $C_{20}H_{28}O_4$ ($M^+-C_8H_{16}O_2$): 332.19875. Found: 332.19291. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Briarellin-H (**8**): Colorless oil: $[\alpha]_D^{26}$ +10.14° (c=2.76, $CHCl_3$). IR (neat): 3472, 1733, 1718, 1652 cm^{-1}. EI-MS m/z (%): 492 (M^+, 1.3), 490 (0.7), 476 (0.9), 458 (0.4), 432 (0.5), 365 (1.6), 348 (2.9), 330 (2.7), 305 (1.9), 127 (57.0), 57 (100). HR-EI-MS, Calcd for $C_{28}H_{44}O_7$ (M^+), 492.30870. Found: 492.31037. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Briarellin-I (**9**): Colorless oil: $[\alpha]_D^{26}$ −35.19° (c=0.97, $CHCl_3$). IR (neat): 3466, 3068, 1734 cm^{-1}. EI-MS m/z (%): 462 (M^+, 0.3), 332 (15.5), 318 (2.7), 314 (8.2), 255 (12.4), 191 (14.6), 175 (20.6), 124 (46.4), 105 (37.0), 57 (100). HR-EI-MS, Calcd for $C_{28}H_{46}O_5$ (M^+): 462.33452. Found: 462.33296. ^1H- and ^{13}C-NMR, see Tables 1 and 2.

Biological Activity Briarellin-E (**5**), which was the only briarellin assessed for pharmacological activity in the present study, displayed modest *in vitro* cytotoxicity against HeLa cells with an estimated IC_{50}=20.0 μg/ml.

Acknowledgments The assistance of Dr. José E. Garcia, Deborath Parrilla, Miriam Ortiz, Luis A. Ríos, and José J. Morales in specimen collection is gratefully acknowledged. We extend our sincere appreciation to the crew of the R/V Isla Magueyes for their assistance during a trip to Mona Island, Puerto Rico, to Dr. Fernando González for cytotoxicity assay of briarellin-E on HeLa cells and to Miss Noralyz Martínez for assistance during the extraction and isolation procedures. HR-EI-MS, HR-FAB-MS and LR-FAB-MS spectral determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Facility (Grant No. CHE8211164). This study was supported by the NSF-EPSCoR (Grant No. R118610677), NIH-MBRS (Grant No. S06RR08102-17), NSF-MRCE (Grant No. R11-8802961), and the University of Puerto Rico FIPI Programs. O. M. Cóbar thanks the University of San Carlos, Guatemala, and the United States Agency for International Development (USAID) for a pre-doctoral fellowship.

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