Milolides G−N, New Briarane Diterpenoids from the Western Pacific Octocoral *Briareum stechei*

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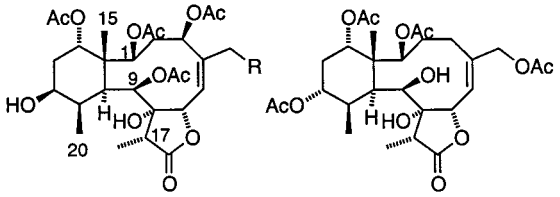
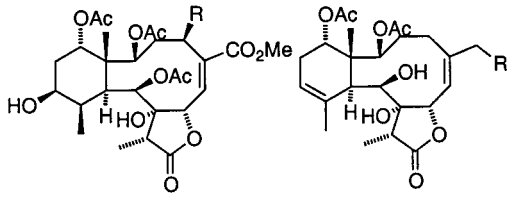
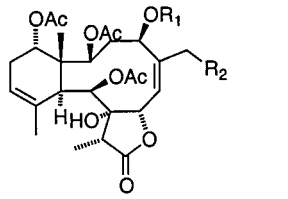
Twelve new briarane diterpenes, designated milolides (**1**−**12**), and one known diterpene, 9-deacetylstylatulide lactone (**13**), were isolated from the Micronesian octocoral *Briareum stechei* collected at Yap, Federated States of Micronesia. Their structures were determined from spectral data.

Numerous diterpenoids of the briarane skeletal class have been obtained from octocorals of the genus *Briareum* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Briaeidae).[1] The most common species studied have been *B. asbestinum*[2−6] and *B. polyanthes*[7] from the tropical western Atlantic and *B. stechei*[8,9] and *B. excavatum* [10−12] from the Indo-Pacific. Some *Briareum* species have also been reported under the synonym *Solenopodium*.[13−16] Earlier we have reported on diterpenoids from *B. asbestinum* from the Caribbean,[2] *S. excavatum* from New Guinea,[15] *S. stechei* from the Great Barrier Reef,[14] and most recently from *B. stechei*,[9] collected at Yap Island, Federated States of Micronesia. From this last source 11 new briaranes, designated milolides, were isolated, one of which had a rearranged carbon skeleton. Further analysis of this rich extract has yielded 12 additional new briarane-type diterpenoids plus one known one. Herein we report the structures of these new briaranes.







8	R_1 = Ac	R_2 = H
9	R_1 = Ac	R_2 = OH
10	R_1 = Ac	R_2 = OAc
11	R_1 = COC_3H_7	R_2 = H
12	R_1 = COC_3H_7	R_2 = OAc

Results and Discussion

Frozen specimens of *B. stechei* were freeze-dried and then subjected to extraction and solvent partitioning.[9] The CH_2Cl_2 solubles were chromatographed over SiO_2, and selected fractions were rechromatographed on reversed-phase C_{18} HPLC and Si gel chromatography to yield compounds **1**−**13**. The known compound 9-deacetylstylatulide lactone (**13**) was identified by comparison of its spectral data with literature values.[17] All 12 new compounds (**1**−**12**) possessed a briarane-type skeleton, and all appeared closely related to **13** and the previously reported milolides[9] by comparative NMR analysis.

Compound **1** (milolide G), an amorphous powder, was assigned the molecular formula $C_{28}H_{40}O_{12}$ on the basis of HRESIMS and NMR data. The NMR data supported the existence of four acetates, a vinyl methyl (δ_H 2.19/δ_C 25.8), two secondary methyls (δ_H 1.19 and 1.11), a tertiary methyl (δ_H 1.09), a γ-lactone (δ_C 176.5), and seven oxygenated carbons (Tables 1 and 2). The COSY, HMQC, and HMBC (Table 1S, Supporting Information) data revealed that **1** has the same structure as milolide A[9] except that the 11,12-epoxide moiety in the latter has been replaced by the 11-methyl, 12-hydroxy group in **1**. Acetate moieties were assigned to C-9 and C-14 because of their HMBC correlations (Table 1S) and to C-2 and C-4 because the chemical shifts of H-2 and H-4 matched closely those of milolide A and related milolides.[9] The more upfield chemical shifts for H/C-12 (δ 4.01/66.9) are consistent with the presence of a hydroxyl at C-12. The relative stereochemistry of all stereocenters except C-11 and C-12 of milolide G (**1**) was confirmed to be the same as that of milolide A by comparison of proton chemical shifts, coupling constants, and NOE correlations for **1** with those of milolide A and other related compounds.[9−11,14] The hydroxyl group at C-12 was assigned the β-configuration primarily because of the NOE noted between H-10 and H-12 (Table 2S, Supporting Information). The methyl group at C-11 was assigned the β-configuration because of the high-field chemical shift of C-20 (δ 9.0), which corresponds to that observed for brianolide,[18] which has the 11β-methyl,12β-OH arrangement. Thus, milolide G was assigned structure **1**.

16-Acetoxymilolide G (**2**) was assigned the molecular formula $C_{30}H_{42}O_{14}$ on the basis of HRESIMS and NMR

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Table 1. ^{1}H NMR Data for Compounds **1**−**7**[a]

H	1	2	3	4	5	6	7
2	4.80 (d, 7.5)	4.75 (d, 7.5)	4.99 (d, 7.5)	4.84 (d, 7)	4.71 (d, 8)	4.87 (d, 7.5)	4.8 (d, 7.5)
3β	3.08 (dd, 14.5, 13)	3.11 (dd, 15.5, 12.5)	2.82 (brt, 14)	2.66 (m)	2.86 (dd, 15.5, 12.5)	2.99 (m)	2.97 (m)
α	1.85 (m)	1.86 (m)	1.51 (m)	1.60 (m)	2.06 (m)	1.56 (m)	1.58 (m)
4	5.09 (dd, 13, 5.8)	5.01 (dd, 12.5, 5.8)	2.44 (br d, 14)	2.63 (m)	5.78 (dd, 12.5, 6.3)	2.44 (br d, 15)	2.52 (br d, 14)
			1.80 (m)	2.44 (m)		1.84 (m)	2.02 (m)
6	5.53 (d, 10)	5.55 (d, 10)	5.48 (d, 9.5)	6.59 (d, 9.5)	6.81 (d, 10)	5.48 (d, 10.5)	5.76 (d, 10.5)
7	5.48 (d, 10)	5.48 (d, 10)	5.51 (d, 9.5)	5.18 (d, 9.5)	5.51 (d, 10)	5.52 (d, 10.5)	5.47 (d, 10.5)
9	5.31 (d, 2.5)	5.29 (d, 2.5)	3.88 (br d, 5.5)	5.28 (br s)	5.30 (br s)	4.58 (br s)	4.61 (dd, 6.5, 3)
10	2.56 (dd, 5, 2.5)	2.50 (dd, 5, 2.5)	2.58 (br d, 4)	2.60 (br s)	2.64 (dd, 4, 2)	2.63 (br s)	2.61 (br s)
11	2.05 (m)	2.02 (m)	1.87 (m)	2.05 (m)	2.03 (m)		
12	4.01 (m)	4.01 (m)	4.76 (br s)	4.02 (m)	4.01 (m)	5.39 (d, 5.5)	5.42 (d, 5)
13	1.80 (m)	1.79 (m)	2.0 (m)	1.77 (m)	1.77 (m)	2.28 (br d 18.5)	2.28 (br d, 18)
	1.75 (m)	1.72 (m)	1.93 (m)	1.77 (m)	1.77 (m)	1.96 (m)	1.97 (m)
14	4.78 (br t, 3)	4.76 (br s)	4.71 (br s)	4.76 (br s)	4.76 (br s)	4.78 (br s)	4.78 (br s)
15	1.09 (s)	1.08 (s)	1.26 (s)	1.08 (s)	1.09 (s)	1.15 (s)	1.15 (s)
16	2.19 (s)	5.32 (dd, 16, 1.3)	4.91 (d, 14.5)			4.93 (d, 15.5)	4.34 (d, 12.5)
		4.74 (dd, 16, 1.3)	4.54 (d, 14.5)			4.52 (d, 15.5)	4.28 (d, 12.5)
17	2.45 (q, 7)	2.44 (q, 7)	3.05 (q, 7)	2.46 (q, 7.5)	2.50 (q, 7)	3.16 (q, 7.5)	3.18 (q, 7.5)
18	1.19 (d, 7)	1.17 (d, 7)	1.11 (d, 7)	1.22 (d, 7.5)	1.23 (d, 7)	1.21 (d, 7.5)	1.23 (d, 7.5)
20	1.11 (d, 7.5)	1.07 (d, 7)	1.17 (d, 8)	1.04 (d, 7)	1.05 (d, 7)	1.81 (s)	1.81 (s)
OAc	2.22, 2.01	2.21, 2.08, 2.01	2.08, 2.00	2.20, 1.91	2.20, 2.05	2.09, 1.98	2.01, 1.92 (s)
	1.97, 1.95 (s)	1.96, 1.94 (s)	1.95, 1.94 (s)	1.90 (s)	1.92, 1.90 (s)	1.93 (s)	
OMe				3.78 (s)	3.80 (s)		

[a] Spectra were recorded in $CDCl_3$ at 500 MHz, referenced to $CDCl_3$ (δ 7.24); J values (Hz) are in parentheses.

Table 2. ^{13}C NMR Data for Compounds **1**−**7**[a,b]

C	1[c]	2[c]	3[c]	4[c]	5[d]	6[c]	7[e]
1	45.5 (s)	45.4 (s)	45.4 (s)	45.1 (s)	45.2 (s)	44.8 (s)	44.8 (s)
2	73.6 (d)	73.7 (d)	75.4 (d)	75.0 (d)	73.9 (d)	75.5 (d)	75.4 (d)
3	37.1 (t)	37.0 (t)	30.6 (t)	30.8 (t)	37.1 (t)	31.3 (t)	31.4 (t)
4	72.7 (d)	69.3 (d)	24.9 (t)	23.1 (t)	67.8 (d)	25.1 (t)	26.3 (t)
5	145.6 (s)	142.1 (s)	142.6 (s)	139.8 (s)	138.3 (s)	143.3 (s)	144.7 (s)
6	122.3 (d)	122.3 (d)	119.1 (d)	132.4 (d)	137.9 (d)	118.0 (d)	121.8 (d)
7	77.8 (d)	77.4 (d)	79.0 (d)	77.6 (d)	77.2 (d)	77.6 (d)	77.5 (d)
8	81.9 (d)	82.0 (s)	83.8 (s)	82.7 (s)	82.5 (s)	83.1 (s)	83.1 (s)
9	74.6 (d)	74.8 (d)	76.9 (d)	76.0 (d)	75.6 (d)	71.4 (d)	71.5 (d)
10	38.0 (d)	37.9 (d)	33.3 (d)	38.2 (d)	38.1 (d)	41.2 (d)	41.2 (d)
11	44.2 (d)	43.9 (d)	43.1 (d)	44.7 (d)	44.6 (d)	135.3 (s)	135.0 (s)
12	66.9 (d)	66.8 (d)	73.0 (d)	67.1 (d)	67.0 (d)	120.5 (d)	120.8 (d)
13	28.8 (t)	28.7 (t)	24.9 (t)	28.8 (t)	28.7 (t)	26.7 (t)	26.7 (t)
14	76.8 (d)	76.7 (d)	74.7 (d)	76.5 (d)	76.3 (d)	73.7 (d)	73.7 (d)
15	15.2 (q)	15.1 (q)	15.2 (q)	15.1 (q)	15.1 (q)	15.6 (q)	15.7 (q)
16	25.8 (q)	66.4 (t)	68.1 (t)	168.9 (s)	167.9 (s)	67.7 (t)	50.5 (t)
17	43.4 (d)	43.4 (d)	44.2 (d)	43.5 (d)	43.5 (d)	43.9 (d)	44.0 (d)
18	6.4 (q)	6.3 (q)	6.8 (q)	6.5 (q)	6.4 (q)	7.0 (q)	7.1 (q)
19	176.5 (s)	176.5 (s)	178.2 (s)	176.0 (s)	175.9 (s)	177.6 (s)	177.2 (s)
20	9.0 (q)	8.9 (q)	14.8 (q)	8.8 (q)	8.8 (q)	24.5 (q)	24.7 (q)
OAc	170.5, 170.3	171.2, 170.8	170.9, 170.9	170.5, 170.2	170.3, 170.3	171.5, 170.9	171.3, 171.1 (s)
	170.2, 168.7 (s)	170.6, 170.1	170.9, 170.6 (s)	169.4 (s)	169.7, 168.7 (s)	170.6 (s)	
		168.7 (s)					
	21.8, 21.4	21.8, 21.2, 21.0	21.5, 21.5	21.7, 21.3	21.7, 21.2	21.4, 21.2	21.4, 21.2 (q)
	21.1, 21.1 (q)	21.0, 20.9 (q)	21.2, 21.0 (q)	21.0 (q)	21.2, 21.9 (q)	21.0 (q)	
OMe				52.7 (q)	53.0 (q)		

[a] Spectra were recorded in $CDCl_3$ at 125 MHz, referenced to $CDCl_3$ (δ 77). [b]Multiplicities are those implied from DEPT experiments. [c] Assignments made by HMQC and HMBC experiments. [d]Assignments aided by H/H COSY and HMQC experiments. [e] Assignments were by analogy.

data. The ^{1}H and ^{13}C NMR data of **2** were nearly identical to those of **1** except for the absence of H/C signals for the vinyl methyl group and the presence of signals for an additional acetoxy group in **2** (Tables 1 and 2). The NMR signals for the H/C-16 in **1** were replaced by signals for an oxymethylene [δ_H 5.32 (dd, J = 16, 1.3 Hz), 4.74 (dd, J = 16, 1.3 Hz); δ_C 66.4 (t)] in **2**. The additional acetoxy group was fixed at C-16 because of an observed HMBC correlation (H-16/16-OAc). The relative stereochemistry of **2** was confirmed to be the same as that of **1** by comparison of the ^{1}H NMR data for **2** with that of milolide G (**1**), and this result was supported by NOE correlations shown in Table 2S. Thus, 16-acetoxymilolide was assigned structure **2**.

The molecular formula of **3** was established by HRESIMS and NMR data as $C_{28}H_{40}O_{12}$. The ^{1}H and ^{13}C NMR data for **3** were similar to those of **1**, **2**, and 16-acetoxymilolide B.[9] The COSY, HMQC, and HMBC spectra revealed that **3** has the briarane skeleton with oxygenation at the same positions as in **2** except that the C-4 oxymethine in **2** was replaced by a methylene carbon. Two acetate moieties were assigned to C-14 and C-16 on the basis of HMBC correlations (Table 1S). Another was placed at C-2 because the proton chemical shift of H-2 is the same as in all the other milolides reported here, and one was assigned to C-12 because of the downfield shift of H-12 compared to the shift observed for H-12 in **1** and **2**. NMR data for H/C-9 [δ_H 3.88 (br d, J = 5.5 Hz): δ_C 76.9(d)] and COSY and HMBC correlations (H-9/9-OH, 9-OH/C-9, respectively) confirmed the presence of a free OH at C-9. The substituents at C-11 and C-12 in **3** were assigned as β and α,

Table 3. ^{1}H NMR Data for Compounds **8**−**12**[a]

H	8	9	10	11	12
2	4.84 (d, 7.5)	4.81 (d, 8)	4.87 (d, 7.5)	4.85 (d, 7.5)	4.88 (d, 7)
3β	2.88 (dd, 15, 13)	2.97 (dd, 15, 13.5)	2.94 (dd, 15, 13.5)	2.88 (dd, 15, 12.8)	2.94 (dd, 15.5, 13
α	1.92 (m)	1.97 (m)	1.97 (m)	1.95 (m)	1.97 (m)
4	5.0 (dd, 13, 5.3)	4.94 (dd, 13.5, 5.3)	4.95 (dd, 13.5, 5.5)	5.03 (dd, 12.8, 5.3)	4.97 (dd, 13, 5)
6	5.53 (d, 10)	5.90 (d, 10)	5.50 (d, 10)	5.53 (dt, 10, 1.5)	5.48 (d, 10)
7	5.59 (d, 10)	5.60 (d, 10)	5.63 (d, 10)	5.61 (d, 10)	5.64 (d, 10)
9	5.98 (d, 3)	6.02 (d, 3)	6.04 (d, 2.5)	6.0 (d, 3)	6.04 (d, 3)
10	2.89 (br s)	2.94 (br s)	2.81 (br s)	2.86 (br s)	2.79 (br s)
12	5.38 (br d, 5)	5.40 (br d, 5)	5.43 (br d, 4.5)	5.41 (br d, 5)	5.44 (br s)
13	2.18 (m)	2.20 (m)	2.21 (m)	2.20 (m)	2.21 (m)
	1.94 (m)	1.93 (m)	1.95 (m)	1.96 (m)	1.97 (m)
14	4.71 (br s)	4.76 (br s)	4.79 (br s)	4.73 (br s)	4.80 (br s)
15	0.92 (s)	0.95 (s)	0.95 (s)	0.93 (s)	0.95 (s)
16	2.12 (d, 1.5)	4.41 (d, 14.5)	5.24 (dd, 16.3, 1.5	2.14 (d, 1.5)	5.26 (dd, 16, 2)
		4.28 (d, 14.5)	4.78 (dd, 16.3, 1.5)		4.79 (dd, 16, 2)
17	2.46 (q, 7)	2.45 (q, 7)	2.49 (q, 7.5)	2.48 (q, 7.5)	2.48 (q, 7)
18	1.19 (d, 7)	1.23 (d, 7)	1.24 (d, 7.5)	1.22 (d, 7.5)	1.25 (d, 7)
20	1.95 (s)	1.96 (s)	1.96 (s)	1.96 (s)	1.96 (s)
OAc	2.17, 2.02	2.19, 2.04	2.20, 2.11, 2.04	2.17, 2.01	2.19, 2.11
	1.99, 1.91 (s)	2.04, 1.90 (s)	2.02, 1.94 (s)	1.92 (s)[b]	2.02, 1.94 (s)[c]

[a] Spectra were recorded in $CDCl_3$ at 500 MHz, referenced to $CDCl_3$ (δ 7.24); *J* values (Hz) are in parentheses. [b] Butyrate residue: 2.26 (t, 7), 1.61 (m, 7.5), 0.92 (t, 7.5). [c] Butyrate residue: 2.27 (td, 7.5, 1.5), 1.61 (m, 7.5), 0.92 (t, 7.5).

respectively, on the basis of the similarity of the ^{13}C NMR shift for C-20 (δ 14.8) in **3** with that of mililides D and E (δ 13.0) [11β-methyl, 12α-OR] in contrast to the C-20 chemical shift of compounds **1**, **2** (~δ 9.0), and related compounds.[9−11,14,19] These configurations were further supported by NOE correlations in Table 2S (H-10/H-11, H-12/H-20). Thus, mililide H was confirmed to have structure **3**.

Compounds **4** and **5** were obtained as white amorphous powders, and their molecular formulas were established by HRESIMS and NMR data as $C_{27}H_{38}O_{12}$ and $C_{29}H_{40}O_{14}$, respectively. The NMR data of **4** were nearly identical to that of **2** (Tables 1 and 2), the only difference being the absence of the signals for H/C-16 (methylene group) and H/C-4 (acetoxymethine) and the occurrence of resonances associated with a methyl ester [δ_H 3.78 (s, OMe); δ_C 168.9 (C-16), 52.7 (OMe)] and a methylene group (C-4). A hydroxyl group was assigned to C-12 because the NMR data for H/C-12 (δ 4.02/67.1) matched that for H/C-12 in **1** and **2**. The configurations at C-11 and C-12 in **4** were confirmed as β, because the chemical shifts for H/C-11, -12, -13, and -20 were nearly the same with those of compounds **1** and **2**.

The three acetate groups in **4** were assigned to C-2, C-9, and C-14, on the basis of HMBC correlations (Table 1S) of the corresponding protons to carbonyl groups, and the methoxycarbonyl group was attached at C-5 because of HMBC correlations (H-6/C-16). The relative stereochemistry of **4** was proposed to be the same as in **1** by comparison of its NMR data with that of **1**, **2**, and closely related compounds.[19,20] Thus mililide I was confirmed as structure **4**. Mililide J (**5**) exhibited ^{1}H and ^{13}C NMR data quite similar to those of **4** except that the C-4 methylene carbon signal was replaced by that of an oxymethine group in **5**, and there was an additional acetyl group. A hydroxyl and four acetate groups were fixed at C-12, C-2, C-4, C-9, and C-14, respectively, on the basis of the NMR chemical shifts for the oxymethines (Tables 1 and 2) and comparison of its NMR data with that of **4** and literature values for erythrolide J.[19] The relative stereochemistry of **5** was assigned as shown from correspondence of its chemical shifts and coupling constants with those of **1**, **2**, and **4** and NOE correlations shown in Table 2S.

Mililide K (**6**) and L (**7**) were assigned the molecular formulas $C_{26}H_{36}O_{10}$ and $C_{24}H_{33}O_8Cl$, respectively, on the basis of their HRESIMS and NMR data. The NMR data of **6** and **7** were nearly identical with those of the known compound **13**, except for H/C-16 (Tables 1 and 2). The ^{13}C NMR signal for C-16 in **13** was replaced by that of an oxymethylene (δ 67.7) in **6** and a chloromethylene (δ 50.5) in **7**.

The additional acetoxy moiety in **6** compared to **13** was assigned to C-16 because of HMBC correlations (H-16/C-5, C-6) (Table 1S). The presence of a chlorine atom in **7** was evident from the mass spectrum, and it was assigned to C-16 on the basis of the ^{13}C NMR shift (δ 50.5) for a deshielded methylene group. The relative stereochemistries of **6** and **7** were assumed to be the same as in **13** by comparison of their NMR chemical shift and coupling features, and that of **6** was also supported by NOE correlations (Table 2S).

Compounds **8**−**12** were obtained as amorphous solids, and their molecular formulas were established by HRESIMS and NMR data. These compounds exhibited ^{1}H and ^{13}C NMR spectra (Tables 3 and 4) nearly identical to those of **6**, **7**, and closely related compounds in the literature.[17] 1D NMR and COSY experiments confirmed the presence of the same substituted skeletal fragments in all five compounds except for variation at C-16.

Mililide M (**8**), 16-hydroxymililide M (**9**), and 16-acetoxymililide M (**10**) were assigned the structures shown based on the above information. Structure **8** was further supported by HMBC correlations (Table 1S). 16-Hydroxymililide M (**9**) was easily identified as the C-16 hydroxylated form of **8** from comparison of their NMR spectra (Tables 3 and 4), which showed that the vinylmethyl signals for the H/C-16 in **8** were replaced by oxymethylene signals [δ_H 4.41 (d, J = 14.5 Hz), 4.28 (d, J = 14.5 Hz); δ_C 66.8 (t)] in **9**. Compound **10** was identified as the 16-acetoxy analogue of **9** from its ^{1}H and ^{13}C NMR spectra. The relative stereochemistries of **8**−**10** were confirmed to be as shown in the drawings based on common NMR chemical shifts, coupling features, and NOE correlations (Table 2S).

Mililide N (**11**) and 16-acetoxymililide (**12**) exhibited ^{1}H and ^{13}C NMR data nearly identical to those of **8** and **10**, respectively, except that signals for one of the acetates in **8** and **10** were replaced by signals for a butyrate moiety (Tables 3 and 4). The above information was supported by COSY, HMQC, and HMBC experiments (Table 1S). The

Table 4. ^{13}C NMR Data for Compounds **8**−**12**[a,b]

C	**8**[c]	**9**[d]	**10**[d]	**11**[c]	**12**[c]
1	44.8 (s)	44.7 (s)	45.4 (s)	44.7 (s)	44.8 (s)
2	72.6 (d)	73.6 (d)	73.7 (d)	72.7 (d)	72.5 (d)
3	37.7 (t)	37.6 (t)	38.5 (t)	37.7 (t)	37.8 (t)
4	72.2 (d)	70.9 (d)	70.1 (d)	72.1 (d)	68.4 (d)
5	145.8 (s)	146.4 (s)	142.6 (s)	145.3 (s)	142.5 (s)
6	122.1 (d)	126.0 (d)	123.1 (d)	122.3 (d)	121.3 (d)
7	77.7 (d)	77.6 (d)	78.3 (d)	78.1 (d)	76.9 (d)
8	81.5 (s)	81.7 (s)	82.4 (s)	81.5 (s)	81.6 (s)
9	69.4 (d)	69.6 (d)	69.5 (d)	69.4 (d)	69.4 (d)
10	39.8 (d)	39.6 (d)	40.3 (d)	39.7 (d)	39.7 (d)
11	134.2 (s)	134.7 (s)	135.1 (s)	134.4 (s)	134.1 (s)
12	120.7 (d)	120.1 (d)	121.2 (d)	120.5 (d)	120.8 (d)
13	26.3 (t)	26.3 (t)	27.1 (t)	26.3 (t)	26.4 (t)
14	73.1 (d)	73.1 (d)	73.3 (d)	73.1 (d)	72.8 (d)
15	14.4 (q)	14.3 (q)	15.0 (q)	14.4 (q)	14.2 (q)
16	25.9 (q)	66.8 (t)	67.1 (t)	25.7 (q)	66.3 (t)
17	43.6 (d)	43.8 (d)	44.4 (d)	43.7 (d)	43.6 (d)
18	6.9 (q)	6.9 (q)	7.6 (q)	6.8 (q)	7.0 (q)
19	176.1 (s)	176.5 (s)	177.1 (s)	176.6 (s)	175.8 (s)
20	24.6 (q)	24.4 (q)	25.2 (q)	24.5 (q)	24.6 (q)
OAc	171.1, 170.4	171.9, 171.1	172.1, 171.5	171.1	171.3, 170.7, 170.7
	170.3, 169.6 (s)	170.6, 169.6 (s)	171.4, 170.9, 170.3 (s)	170.4, 169.6 (s)	169.5 (s)
	21.6, 21.4, 21.2	21.6, 21.2	22.3, 22.0, 21.8	21.5, 21.4	21.6, 21.2
	21.1 (q)	21.2, 21.1 (q)	21.8, 21.6 (q)	21.1 (q)[e]	21.1, 21.0 (q)[f]

[a] Spectra were recorded in $CDCl_3$ at 125 MHz, referenced to $CDCl_3$ (δ 77). [b] Multiplicities were determined from DEPT experiments. [c] Assignments were made by HMQC and HMBC experiments. [d] Assignments were by analogy. [e] Butyrate residue: 172.9 (s), 36.1 (t), 18.4 (t), 13.6 (q). [f] Butyrate residue: 172.8 (s), 36.1 (t), 18.4 (t), 13.6 (q).

butyrate moiety was located at C-4 in **11** because HMBC correlations shown in Table 1S fixed the acetate groups at C-2, C-9, and C-14. Acetate esters were fixed in compound **12** at C-2, C-4, and C-16 by HMBC data (Table 1S). The oxygen at C-9 must be acetylated because the only acetate methyl ^1H NMR signal not assigned by HMBC occurred at δ 2.19, consistent with the δ 2.17−2.22 shift confirmed by HMBC for the methyl of the acetate groups at C-9 in compounds **1**, **2**, **4**, **8**, and **11**. Thus the butyrate ester in **12** must be at C-4. The stereochemistries of **11** and **12** are assumed to be the same as in **8** and **10** by comparison of their ^1H and ^{13}C NMR data. Thus, milolide N and 16-acetoxymilolide N were assigned structures **11** and **12**, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Rudolph Autopol III automatic polarimeter. FABMS and ESIMS data were obtained on VG ZAB-E and Micromass Q-TOF mass spectrometers, respectively. NMR experiments were performed on a Varian VXR-500 spectrometer equipped with a 3 mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver, using standard Varian software. NMR signals are reported in parts per million (δ), referenced to $CDCl_3$ (δ_H 7.24; δ_C 77). Vacuum flash column chromatography was carried out on Si gel 60 H (Merck) and LRP-2 (Whatman), and preparative HPLC was performed using an RI detector and Phenomenex ODS Prep (250 × 10 mm) column. Si gel 60 (Merck, 230−400 mesh) was used for open column chromatography.

Animal Material. *Briareum stechi* was collected in August 1995 at Mil Channel, Yap, Micronesia. This organism was described fully in a previous paper.[9] A voucher specimen is maintained at the University of Oklahoma (24YA95) and the California Academy of Sciences (CAS #117221).

Extraction and Isolation. Frozen specimens were freeze-dried and then subjected to extraction and solvent partitioning as described previously.[9] A portion (7.0 g) of the CH_2Cl_2 solubles was chromatographed on a Si gel vacuum flash column using stepwise elution with CH_2Cl_2−MeOH (50:1, 30:1, 20:1, 10:1, 5:1, 3:1, and 1:1). Fractions were combined based on their TLC patterns to yield fractions F501−F506. Fractions F501, F502, and F503 were fractionated on a C_{18} reversed-phase Si gel vacuum flash column with an MeOH−H_2O mixture to obtain fractions F520−F528, F507−F514, and F515−F519, respectively. Fraction F515 was rechromatographed over C_{18} reversed-phase HPLC with 47% H_2O−MeOH as eluent to yield compounds **1**, **2**, **4**, and **5** (6.9, 29.2, 4, and 3 mg, respectively).

Compounds **3** and **6** (12 and 8.4 mg, respectively) were obtained by C_{18} reversed-phase HPLC (38% H_2O−MeOH) of fraction F507. Fraction F523 was chromatographed over C_{18} reversed-phase HPLC (33% H_2O−MeOH) to isolate compounds **7**−**10**, **12** (8.2, 123, 55.9, 15.4, and 2.8 mg, respectively), and mixtures of compounds. From one of these mixtures, compounds **11** and **13** (9.3 and 6.9 mg, respectively) were separated by Si gel chromatography using hexanes−EtOAc (1:1.5) as eluent.

Milolide G (1): $[\alpha]_D^{23}$ +53.6° (*c* 0.38, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 569 $[M + H]^+$, 591 $[M + Na]^+$; ESIMS *m/z* 591 $[M + Na]^+$; HRESIMS *m/z* 591.2454 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_{12}Na$, 591.2417).

16-Hydroxymilolide G (2): $[\alpha]_D^{23}$ +67.2° (*c* 0.61, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 649 $[M + Na]^+$; ESIMS *m/z* 649 $[M + Na]^+$; HRESIMS *m/z* 649.2493 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_{14}Na$, 649.2472).

Milolide H (3): $[\alpha]_D^{23}$ +8.6° (*c* 0.62, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 569 $[M + H]^+$, 591 $[M + Na]^+$; ESIMS *m/z* 591 $[M + Na]^+$; HRESIMS *m/z* 591.2438 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_{12}Na$, 591.2417).

Milolide I (4): $[\alpha]_D^{23}$ +3.8° (*c* 0.48, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 555 $[M + H]^+$; ESIMS *m/z* 577 $[M + Na]^+$; HRESIMS *m/z* 577.2313 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_{12}Na$, 577.2261).

Milolide J (5): $[\alpha]_D^{23}$ +34.5° (*c* 0.2, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 613 $[M + H]^+$; ESIMS *m/z* 635 $[M + Na]^+$; HRESIMS *m/z* 635.2308 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_{14}Na$, 635.2316).

Milolide K (6): $[\alpha]_D^{23}$ −20.7° (*c* 0.59, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS *m/z* 531 $[M + Na]^+$; HRESIMS *m/z* 531.2205 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_{10}Na$, 531.2206).

Milolide L (7): $[\alpha]_D^{23}$ −30.6° (*c* 0.46, CH_2Cl_2); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS *m/z* 507 $[M + Na]^+$; HRESIMS *m/z* 507.1828 $[M + Na]^+$ (calcd for $C_{24}H_{33}O_8ClNa$, 507.1762).

Milolide M (8): $[\alpha]_D^{23}$ +31.1° (*c* 0.47, CH_2Cl_2); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4; FABMS *m*/*z* 551 $[M + H]^+$; ESIMS *m*/*z* 573 $[M + Na]^+$; HRESIMS *m*/*z* 573.2363 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_{11}Na$, 573.2312).

16-Hydroxymilolide M (9): $[\alpha]_D^{23}$ +49.6° (*c* 0.27, CH_2Cl_2); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4; FABMS *m*/*z* 567 $[M + H]^+$; 589 $[M + Na]^+$; ESIMS *m*/*z* 589 $[M + Na]^+$; HRESIMS *m*/*z* 589.2286 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_{12}Na$, 589.2261).

16-Acetoxymilolide M (10): $[\alpha]_D^{23}$ +23.9° (*c* 0.18, CH_2Cl_2); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4; FABMS *m*/*z* 631 $[M + H]^+$; ESIMS *m*/*z* 631 $[M + Na]^+$; HRESIMS *m*/*z* 631.2356 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{13}Na$, 631.2367).

Milolide N (11): $[\alpha]_D^{23}$ +61.7° (*c* 0.6, CH_2Cl_2); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4; ESIMS *m*/*z* 601 $[M + Na]^+$; HRESIMS *m*/*z* 601.2650 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_{11}Na$, 601.2625).

16-Acetoxymilolide N (12): $[\alpha]_D^{23}$ +22.2° (*c* 0.17, CH_2Cl_2); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 4; ESIMS *m*/*z* 659 $[M + Na]^+$; HRESIMS *m*/*z* 659.2714 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_{13}Na$, 659.2680).

9-Deacetylstylatulide lactone (13): $[\alpha]_D^{23}$ −3.4° (*c* 0.18, $CHCl_3$); lit.[17] $[\alpha]_D^{26}$ −3° (*c* 0.58, $CHCl_3$).

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Supporting Information Available: HMBC correlations for **1**−**6**, **8**, **11**, and **12** (Table 1S) and selected NOESY correlations for **1**−**3**, **5**, **6**, and **8**−**10** (Table 2S). This information is available free of charge via the Internet at http://pubs.acs.org.

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