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# DITERPENES FROM THE POHNPEIAN MARINE SPONGE *CHELONAPLYSILLA* SP.

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ABSTRACT.—Nine diterpenes have been isolated from the marine sponge *Chelonaplysilla* sp. collected in Pohnpei, Federated States of Micronesia. These include the known diterpenes 1-bromo-8-ketoambliol A acetate [**2**], dendrillolide A [**3**], dendrillolide D [**4**], norrisolide [**5**], 12-desacetoxyshahamin C [**6**], and aplyviolene [**7**]. Three novel rearranged spongian diterpenes, chelonaplysins A [**8**], B [**9**], and C [**10**], were identified by interpretation of spectral data and chemical correlation with known compounds. Aplyviolene [**7**], chelonaplysin B [**9**], and chelonaplysin C [**10**] exhibited antimicrobial activity against the bacterium *Bacillus subtilis*. The secondary metabolite composition of this collection of *Chelonaplysilla* sp. is compared to the composition previously reported for samples from Palau.

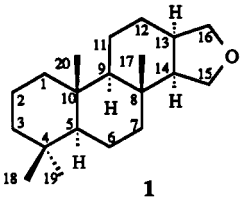
In a continuation of our survey of the chemistry of marine sponges of the order Dendroceratida (1,2), we have investigated the secondary metabolite composition of an unidentified species of the genus *Chelonaplysilla* de Laubenfels collected in Pohnpei, Federated States of Micronesia. Previous studies (2,3) of the secondary metabolites of a *Dendrilla* sp. collected in Palau had resulted in the isolation and characterization of ten diterpenoid compounds, eight of which are presumably derived from the spongian diterpene skeleton **1**. This Palauan *Dendrilla* sp. has now been reidentified as *Chelonoplysilla* sp. de Laubenfels,[1] and we believe from field identifications that the two specimens of *Chelonaplysilla* are of the same, as yet unidentified, species. In this paper we report the reisolation of five of the ten diterpenoids previously isolated from the Palauan specimen: 1-bromo-8-ketoambliol A acetate [**2**], dendrillolide A [**3**], dendrillolide D [**4**], norrisolide [**5**], and 12-desacetoxyshahamin C [**6**], along with the known compound aplyviolene [**7**] (4,5) and three new rearranged spongian diterpenes, chelonaplysins A [**8**], B [**9**], and C [**10**].

## RESULTS AND DISCUSSION

The purple dendroceratid sponge *Chelonaplysilla* sp. was collected from numerous locations in coral reef habitats around Pohnpei, Federated States of Micronesia. The frozen sponge was lyophilized and extracted with hexane, $CH_2Cl_2$, and EtOAc to yield a crude non-polar extract which showed antimicrobial activity against the bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Flash chromatography on silica followed by repeated hplc separations on μ-Partisil yielded chelonaplysin A [**8**], 1-bromo-8-ketoambliol A acetate [**2**], dendrillolide A [**3**], chelonaplysin B [**9**], aplyviolene [**7**], norrisolide [**5**], chelonaplysin C [**10**], 12-desacetoxyshahamin C [**6**], and dendrilloide D [**4**], in order of increasing retention time on μ-Partisil. The identities of the known compounds were determined by comparison of spectral data, usually $^1$H-nmr data, with published values (1–6).

Chelonaplysin A [**8**] was isolated as a clear oil that was determined to have a molecular formula of $C_{27}H_{40}O_7$ by analysis of the $^{13}$C-nmr data and high resolution mass measurement of the fragment ion at *m/z* 416.2567 (32%) $[M - HOAc]^+$. The facile loss of HOAc in the ei mass spectrum along with a $^1$H-nmr resonance at δ 2.07 (s, 3H) indicated the presence of an acetate group. Additional fragments in the mass spec-

[1]P.R. Bergquist, personal communication.



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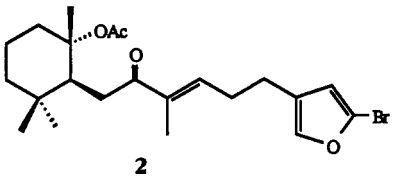

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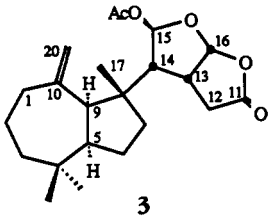

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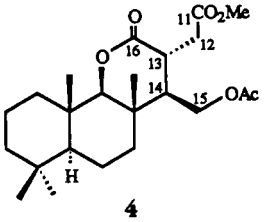

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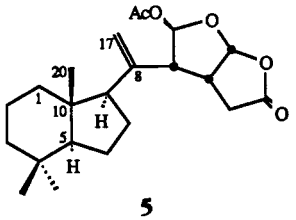

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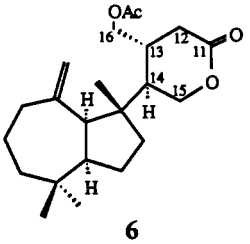

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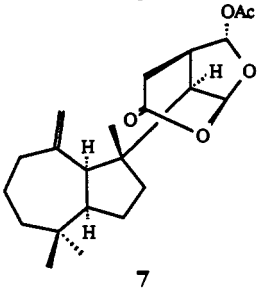

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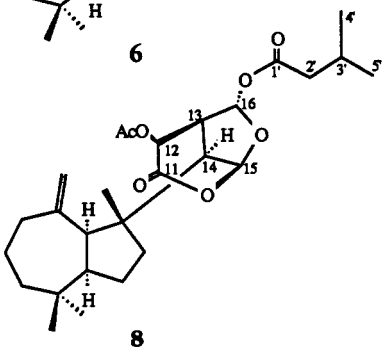

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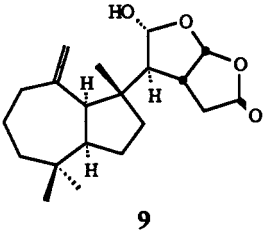

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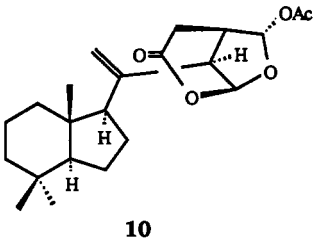

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trum at *m/z* 314 (25%) $[M-HOAc-C_5H_{10}O_2]^+$, 191 (8%) $[C_{14}H_{23}]^+$, and 137 (100%) $[C_{10}H_{17}]^+$ indicated the presence of isovalerate and perhydroazulene moieties, respectively. The presence of an isovalerate unit was confirmed by the observation of an isolated nine-proton spin system in the $^1$H-nmr spectrum. Two mutually coupled proton signals at δ 2.37 (dd, 1H, $J$ = 14.4, 7.5 Hz, H-2′) and 2.31 (dd, 1H, $J$ = 14.4, 6.8 Hz, H-2′) were assigned to a methylene unit adjacent to a carbonyl carbon. These signals were coupled to a methine proton signal at δ 2.12 (m, 1H, $J$ = 7.5, 6.8, 6.5 Hz, H-3′), which was further coupled to a six-proton doublet at 0.97 ($J$ = 6.5 Hz, Me-4′, -5′). The presence of the perhydroazulene hydrocarbon ring system was confirmed by the observation of characteristic $^1$H-nmr chemical shifts and coupling constants of $CH_2$-1, H-9, and $CH_2$-20 and comparison of the $^{13}$C-nmr data with those reported for

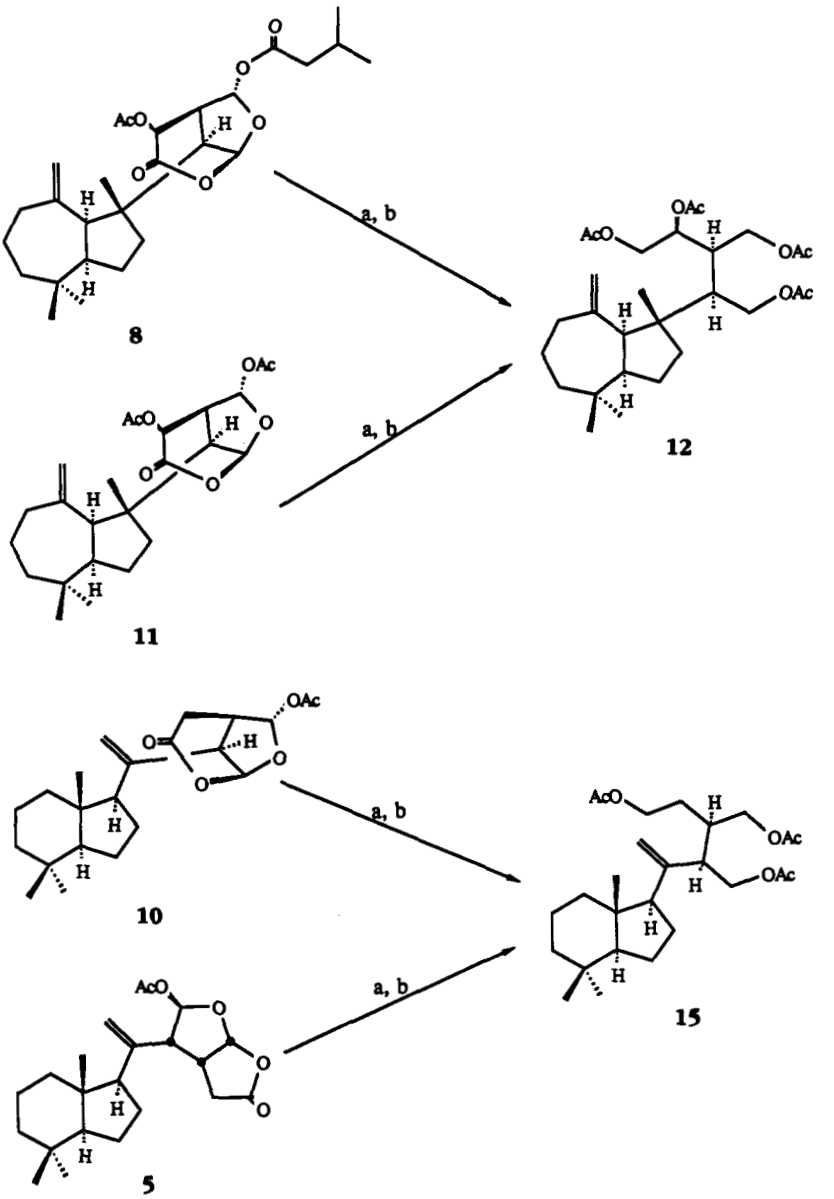
macfarlandin E [**11**] (7). The remaining signals in the $^1$H- and $^{13}$C-nmr spectra of chelonaplysin A [**8**] were observed to have chemical shifts and coupling constants nearly identical to those assigned to the [3.2.1] oxabicyclic ring system in macfarlandin E (Table 1). Ir bands at 1775 and 1755 cm$^{-1}$ substantiated the presence of a δ-lactone ring with an ester unit at the α position (8). The acetate group was placed at the α position when an nOe was observed at δ 2.12 (H-3′, 11.8%) upon irradiation at 2.63 (dd, 1H, $J$ = 3.9, 2.5 Hz, H-14). This result placed the isovalerate group at C-16, leaving the acetate to be attached at C-12. The signals at δ 5.70 (dd, 1H, $J$ = 2.5, 1.1 Hz, H-15, 11.3%) and 2.82 (ddd, 1H, $J$ = 5.0, 3.9, 1.1, H-13, 4.6%) were also enhanced upon irradiation of H-14, indicating the remaining stereochemistry about the five-membered ring.

TABLE 1. Comparison of $^1$H-nmr Data [360 MHz, ($CDCl_3$), chemical shift (δ), multiplicity, coupling constants (Hz)] of the Oxabicyclic Ring Portions of Macfarlandin E [**11**], Macfarlandin D [**14**], Chelonaplysin A [**8**], and Chelonaplysin C [**10**].

| Proton | Compound | | | |
|---|---|---|---|---|
| | **8** | **11** | **10** | **14** |
| H-12α | 5.80, d, 5.0 | 5.80, d, 5.0 | 3.12, dd, 19.2, 6.2 | 3.12, dd, 20, 6.2 |
| H-12β | | | 2.59, dd, 19.2, 0.5 | 2.64, d, 20 |
| H-13 | 2.82, ddd, 5.0, 3.9, 1.1 | 2.87, ddd, 5.0, 3.8, 0.8 | 2.78, m, 6.2, 3.5, 1.1, 0.5 | 2.62, m, 6.2, 3.1, 1.2 |
| H-14 | 2.63, dd, 3.9, 2.5 | 2.67, dd, 3.8, 2.5 | 3.18, m, 3.5, 3.4, 2.2, 0.9 | 2.45, dd, 3.1, 3.1 |
| H-15 | 5.70, dd, 2.5, 1.1 | 5.73, dd, 2.5, 0.8 | 5.85, dd, 3.4, 1.1 | 5.75, dd, 3.1, 1.2 |
| H-16 | 6.47, s | 6.49, s | 6.24, s | 6.13, s |

The structure proposed for chelonaplysin A [**8**] from the interpretation of spectral data was verified by chemical correlation with macfarlandin E [**11**] (Scheme 1). Chelonaplysin A [**8**] was reduced with $LiAlH_4$ to cleave both esters, open the lactone ring, and cleave the remaining acetal bond. The resulting tetraol was acetylated with $Ac_2O$ in pyridine to produce the tetraacetate **12**. This tetraacetate was identical by tlc, $^1$H nmr, and lrms to the tetraacetate prepared from marfarlandin E [**11**] under similar conditions (1). This procedure also verified the stereochemistry assigned to chelonaplysin A [**8**].

Chelonaplysin B [**9**] was isolated as a clear oil that was found to have a molecular formula of $C_{20}H_{30}O_4$ by high resolution mass measurement of the molecular ion ($m/z$ 334.2136). The base peak fragment in the chemical ionization mass spectrum ($NH_3$) at $m/z$ 317 $[M-OH]^+$ and the presence of a $D_2O$ exchangeable signal in the $^1$H-nmr spectrum at δ 2.79 (br, 1H) indicated the presence of an alcohol group. A perhydroazulene hydrocarbon unit was suggested from the observation of the characteristic fragment in the mass spectrum at $m/z$ 191 $[C_{14}H_{23}]^+$ (7%) and $^1$H-nmr resonances at δ 4.81 (d, 1H, $J$ = 2.0 Hz, H-20), 4.60 (d, 1H, $J$ = 2.0 Hz, H-20′), 2.53 (d, 1H, $J$ = 8.4 Hz, H-9), and 2.33 (br dd, 1H, $J$ = 11.9, 4.7 Hz, H-1). A band at 1785 cm$^{-1}$ in the ir spectrum was assigned to a γ-lactone carbonyl stretch while the expected OH stretch was not observed, indicating that the alcohol group was strongly hydrogen-bonded. This alcohol group was determined to be a part of a hemiacetal unit incorporated in a [3.3.0] oxabicyclic ring system by analysis of the data from $^1$H-$^1$H COSY experiment. The acetal proton signal at δ 6.05 (d, 1H, $J$ = 5.9 Hz, H-16) was coupled to



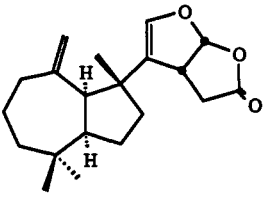

SCHEME 1. Chemical correlation of chelonaplysin A [**8**] with macfarlandin E [**11**] and chelonaplysin C [**10**] with norrisolide [**5**]. (a) $LiAlH_4$, $Et_2O$; (b) $Ac_2O$, pyridine.

a methine proton signal at 3.07 (m, 1H, $J$ = 10.7, 5.9, 3.8, 1.7 Hz, H-13) which was further coupled to three signals [2.92 (dd, 1H, $J$ = 17.9, 10.7 Hz, H-12β), 2.70 (dd, 1H, $J$ = 17.9, 3.8 Hz, H-12α), and 2.22 (d, 1H, $J$ = 1.7 Hz, H-14)]. The H-14 signal was not observed to be coupled to the hemiacetal signal at δ 5.49 (s, 1H, H-15), indicating an H-14–C-14–C-15–H-15 torsional angle of approximately 90°.

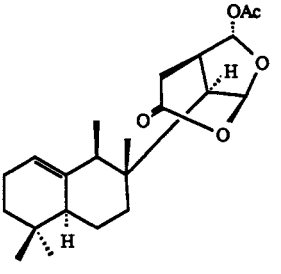
This interpretation, along with further stereochemical assignments of chelonaplysin B [**9**], was confirmed by the results from nuclear Overhauser effect difference spectroscopy (NOEDS) experiments. Irradiation of the H-13 signal at δ 3.07 (m) resulted in an enhancement in the resonance of H-16 (6.7%), verifying the cis fusion of the

oxabicyclic ring system. Irradiation of the signal at δ 2.70 (H-12α) produced an enhancement in the signal at 2.22 (H-14, 5.7%) indicating the proximal arrangement of these two protons. The zero coupling between H-14 and H-15 and the small coupling constant between H-14 and H-13 ($J_{13,14}$ = 1.7 Hz) indicated that H-14 is in a trans orientation to these two adjacent protons. Although these small coupling constants appear unusual with respect to other compounds in this series, there is a conformation of the [3.3.0] oxabicyclic ring system with a hydrogen bond between the hydroxyl hydrogen and the lactone oxygens that has H-14–C-14–C-13–H-13 and H-14–C-14–C-15–H-15 torsional angles sufficiently close to 90° to account for the observed coupling constants.

The relative stereochemistry of chelonaplysin B [**9**] at C-14 and C-15 is opposite to that found in other rearranged spongian diterpenes possessing the [3.3.0] oxabicyclic ring system (e.g., dendrillolide A [**3**] and norrisolide [**5**]). Therefore, we propose that chelonaplysin B [**9**] may be derived via hydration of the C-14/C-15 double bond of dendrilloide C [**13**], rather than by the hydrolysis of the acetate in dendrillolide A [**3**]. Dendrilloide C [**13**] was not isolated from this collection of *Chelonaplysilla* sp., but it has been reported from two samples of the same species collected in Palau (2,3).



**13**



**14**

Chelonaplysin C [**10**] was isolated as a white solid, mp 148°, which displayed a molecular ion at *m/z* 376.2261 ($C_{22}H_{32}O_5$) in the high resolution mass spectrum. The presence of δ-lactone and acetate moieties was suggested by an ir band at 1755 $cm^{-1}$, a $^1$H-nmr resonance at δ 2.08 (s, 3H), and $^{13}$C-nmr resonances at δ 169.4 (s), 166.8 (s), and 21.0 (q). The presence of δ-lactone and acetate units, along with the observation of two acetal carbons in the $^{13}$C-nmr spectrum [δ 101.2 (d) and 100.6 (d)], suggested that a (3.2.1] oxabicyclic ring system was present in chelonaplysin C [**10**]. Comparison of the chemical shifts and coupling constants in the $^1$H-nmr spectrum of chelonaplysin C with those assigned to the [3.2.1] oxabicyclic ring system of macfarlandin D [**14**] (7) confirmed that chelonaplysin C possessed this unit (Table 1). Allylic couplings between the terminal methylene proton signal at δ 5.21 (dd, 1H, $J$ = 2.1, 0.9 Hz, H-17) and the signals at 3.18 (m, 1H, $J$ = 3.5, 3.4, 2.2, 0.9 Hz, H-14) and 2.14 (m, 1H, H-9) further extended the contiguous spin system of the oxabicyclic ring system and suggested a hydrocarbon moiety similar to that found in norrisolide [**5**]. The presence of the 6/5 bicyclic hydrocarbon unit was further supported by comparison of the $^{13}$C-nmr data of chelonaplysin C [**10**] to those of norrisolide [**5**] (6).

The structure of chelonaplysin C [**10**] was verified and the stereochemistry assigned by chemical correlation with norrisolide [**5**], whose structure is known from single-crystal X-ray diffraction studies (6). Chelonaplysin C was treated with $LiAlH_4$ to reduce the acetate, open the lactone ring, and cleave the remaining acetal bond. The resulting triol was acetylated with $Ac_2O$ in pyridine to yield the triacetate **15**. Treatment of norrisolide in a similar manner yielded a triacetate **15** which was identical by tlc, $^1$H nmr, and lrms (Scheme 1).

It is interesting to note the slight differences between the secondary metabolite composition of collections of this *Chelonaplysilla* sp. now reported in the literature (Table 2). The major diterpene in both Palauan collections (2,3) was dendrilloide A [**3**], while this compound was found only as a very minor metabolite in our sample from Pohnpei. Conversely, aplyviolene [**7**] was the major metabolite in our Pohnpei collection, but was not reported from either of the Palauan samples. Chelonaplysins A [**8**], B [**9**], and C [**10**] were isolated from our Pohnpeian sample in quantities greater than those reported for the minor metabolites of the Palauan samples and are therefore believed to be new metabolites from the Pohnpei collection of *Chelonaplysilla* sp. rather than simply minor components which were overlooked in the previous investigations. These differences may be an indication that these collections of *Chelonaplysilla* sp. represent two different species, but we believe from our field observations that this is not the case. We prefer the explanation that the difference in chemistry of these samples is due to temporal or environmental factors, or that the Pohnpeian and Palauan populations of this *Chelonaplysilla* sp. are chemically distinct. Additional collections will be necessary to verify this.

TABLE 2. Concentration of Diterpenes in Different Collections of *Chelonaplysilla* sp. (in percent dry wt).

| Compound | Collection | | |
|---|---|---|---|
| | 1984 Palau | 1988 Palau | 1989 Pohnpei |
| Dehydroambliol A | 0.005% | 0.005% | — |
| 1-Bromo-8-ketoambliol A acetate [**2**] | 0.34%[a] | 0.11% | 0.13% |
| Norrisolide [**5**] | 0.01% | 0.061% | 0.058% |
| Dendrillolide A [**3**] | 0.31%[a] | 0.63%[a] | 0.012% |
| Dendrillolide B | 0.004% | — | — |
| Dendrillolide C | 0.008% | 0.004% | — |
| Dendrillolide D [**4**] | — | 0.01% | 0.088% |
| Dendrillolide E | — | 0.009% | — |
| 12-Desacetoxypolyrhaphin A | — | 0.006% | — |
| 12-Desacetoxyshahamin C [**6**] | — | 0.007% | 0.10% |
| Aplyviolene [**7**] | — | — | 0.55%[a] |
| Chelonaplysin A [**8**] | — | — | 0.095% |
| Chelonaplysin B [**9**] | — | — | 0.006% |
| Chelonaplysin C [**10**] | — | — | 0.096% |

[a]Major compound(s) in each collection.

Aplyviolene [**7**] and chelonaplysin C [**10**] exhibited antimicrobial activity against the Gram positive bacterium *B. subtilis* at a concentration of 5 μg/disk in the standard disk assay. Chelonaplysin B [**9**] was active against *B. subtilis* at 50 μg/disk.

## EXPERIMENTAL

EXTRACTION AND CHROMATOGRAPHY OF *CHELONAPLYSILLA* SP.—Specimens of *Chelonaplysilla* sp. (SIO Benthic Invertebrate Collection #P1112) were collected by hand from rock and coral substrates at various depths (2 to 25 m) and locations around Pohnpei and Ant Atoll, Federated States of Micronesia. The thinly encrusting, fleshy and fragile, dark purple sponge *Chelonaplysilla* sp. is an aplysillid sponge having a fiber structure similar to that of *Aplysilla*, but possessing a dermis conspicuously reinforced by a fine reticulum of sand. The combined collections were stored frozen at −10° for 1 month and then freeze-dried. The lyophilized sponge tissue (16.8 g) was extracted with hexane (3 × 200 ml), $CH_2Cl_2$ (3 × 200 ml), and EtOAc (1 × 200 ml) to yield a thick yellow oil (619 mg) that exhibited antimicrobial activity against *S. aureus* and *B. subtilis*. This combined extract was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, bed size 31.5 × 3.0 cm) using a solvent gradient from hexane-EtOAc (9:1) to hexane-EtOAc (1:9) to elute the mixture. This procedure yielded aplyviolene [**7**] (92.5 mg, 0.55% dry weight)

and dendrillolide D [**4**] (14.8 mg, 0.088% dry wt) along with two fractions which appeared by $^1$H nmr to contain mixtures of spongian diterpenes.

The less polar of these two fractions (99 mg) was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, bed size 14.5 × 1.5 cm) using a solvent gradient from hexane to hexane-$Et_2O$ (1:4) to elute the mixture. This yielded 1-bromo-8-ketoambliol A acetate [**2**] (21.3 mg, 0.13% dry wt) and two additional mixtures. The first mixture (27 mg) was separated by hplc on μ-Partisil using hexane-$Et_2O$ (3:1) as eluent to yield chelonaplysin A [**8**] (16.0 mg, 0.095% dry wt). The second mixture (12 mg) was separated by hplc on μ-Partisil using hexane-EtOAc (4:1) as eluent to yield dendrillolide A [**3**] (2.1 mg, 0.012% dry wt).

The more polar spongian mixture (75 mg) was separated by hplc on μ-Partisil using hexane-$Et_2O$ (45:55) to elute the components. This yielded chelonaplysin B [**9**] (1.0 mg, 0.006% dry wt), norrisolide [**5**] (9.8 mg, 0.058% dry wt), chelonaplysin C [**10**] (16.2 mg, 0.096% dry wt), and 12-desacetoxyshahamin C [**6**] (17.5 mg, 0.10% dry wt).

CHELONAPLYSIN A [**8**].—Clear oil; $[\alpha]_D$ −45.8° ($c$ = 0.24, $CHCl_3$); ir ($CHCl_3$) 1775, 1755, 1630, 1370, 1220 cm$^{-1}$; $^1$H nmr ($CDCl_3$) δ 0.92 (s, 3H), 0.97 (s, 3H), 0.97 (d, 6H, $J$ = 6.5 Hz, C$H_3$-4′, -5′), 1.06 (s, 3H), 2.07 (s, 3H, OCOC$H_3$), 2.12 (m, 1H, $J$ = 7.5, 6.8, 6.5 Hz, H-3′), 2.31 (dd, 1H, $J$ = 14.4, 6.8 Hz, H-2′), 2.37 (dd, 1H, $J$ = 14.4, 7.5 Hz, H-2′), 2.38 (br dd, 1H, $J$ = 12.5, 5.1 Hz, H-1), 2.63 (dd, 1H, $J$ = 3.9, 2.5 Hz, H-14), 2.72 (d, 1H, $J$ = 8.5 Hz, H-9), 2.82 (ddd, 1H, $J$ = 5.0, 3.9, 1.1 Hz, H-13), 4.65 (d, 1H, $J$ = 1.7 Hz, H-20), 4.87 (d, 1H, $J$ = 1.7 Hz, H-20), 5.70 (dd, 1H, $J$ = 2.5, 1.1 Hz, H-15), 5.80 (d, 1H, $J$ = 5.0 Hz, H-12), 6.47 (s, 1H, H-16); $^{13}$C nmr ($CDCl_3$) [$^{13}$C-nmr assignments made by comparison of the data to that of known compounds (1,2,7)] δ 21.0 (q, OCOC$H_3$), 22.3 (q, 2C, C$H_3$-4′, -5′), 24.4 (q, C-17), 25.6 (d, C-3′), 25.9 (q, C-18), 27.0 (t, C-6), 28.2 (t, C-2), 34.2 (q, C-19), 36.0 (s, C-4), 37.5 (t), 37.9 (t), 38.3 (t), 42.7 (t, C-2′), 44.4 (d, C-13), 45.4 (s, C-8), 51.8 (d, C-14), 54.2 (d, C-5), 57.7 (d, C-9), 65.8 (d, C-12), 96.0 (d, C-16), 101.1 (d, C-15), 115.2 (t, C-20), 152.5 (s, C-10), 165.8 (s, C-11), 169.2 (s, OCOMe), 171.3 (s, C-1′); hrms (ei) $m/z$ [M − HOAc]$^+$ 416.2567 ($C_{25}H_{36}O_5$ requires 416.2563); eims $m/z$ (rel. int.) [M − HOAc]$^+$ 416 (32%), [M − HOAc − $C_5H_{10}O_2$]$^+$ 314 (25%), 216 (25%), 215 (33%), 150 (25%), [$C_{10}H_{17}$]$^+$ 137 (100%).

CHELONAPLYSIN B [**9**].—Clear oil; $[\alpha]_D$ +4.5° ($c$ = 0.11, $CHCl_3$); ir ($CHCl_3$) 1785, 1370, 1220 cm$^{-1}$; $^1$H nmr ($CDCl_3$) δ 0.79 (s, 3H), 0.93 (s, 3H), 0.97 (s, 3H), 1.93 (ddd, 1H, $J$ = 11.2, 8.7, 8.4 Hz, H-5), 2.22 (d, 1H, $J$ = 1.7 Hz, H-14), 2.33 (br dd, 1H, $J$ = 11.9, 4.7 Hz, H-1), 2.53 (d, 1H, $J$ = 8.4 Hz, H-9), 2.70 (dd, 1H, $J$ = 17.9, 3.8 Hz, H-12α), 2.79 (br, 1H, $D_2O$ exchangeable, O$H$), 2.92 (dd, 1H, $J$ = 17.9, 10.7 Hz, H-12β), 3.07 (m, 1H, $J$ = 10.7, 5.9, 3.8, 1.7 Hz, H-13), 4.60 (d, 1H, $J$ = 2.0 Hz, H-20), 4.81 (d, 1H, $J$ = 2.0 Hz, H-20′), 5.49 (s, 1H, H-15), 6.05 (d, 1H, $J$ = 5.9 Hz, H-16); $^{13}$C nmr ($CDCl_3$) [$^{13}$C-nmr assignments made by comparison of the data to that of known compounds (1,2,7)] δ 21.2 (q, C-17), 25.7 (q, C-18), 26.3 (t, C-6), 28.8 (t, C-2), 29.7 (t, C-12), 31.2 (s, C-4), 34.4 (q, C-19), 36.9 (t), 37.7 (t), 38.5 (t), 40.2 (d, C-13), 48.0 (s, C-8), 54.4 (d, C-5), 56.6 (d, C-9), 66.1 (d, C-14), 103.2 (d, C-16), 109.5 (d, C-15), 114.5 (t, C-20), 154.0 (s, C-10), 168.8 (s, C-11); hrms (ci, $NH_3$) $m/z$ [M]$^+$ 334.2136 ($C_{20}H_{30}O_4$ requires 334.2144); cims ($NH_3$) $m/z$ (rel. int.) [M + $NH_4$]$^+$ 352 (94%), [M]$^+$ 334 (26%), [M − OH]$^+$ 317 (100%), [$C_{14}H_{23}$]$^+$ 191 (7%).

CHELONAPLYSIN C [**10**].—White flakes from hexane/$Et_2O$; mp 148° $[\alpha]_D$ −6.1° ($c$ = 0.23, $CHCl_3$); ir ($CHCl_3$) 1755, 1640, 1370, 1360, 1225 cm$^{-1}$; $^1$H nmr ($CDCl_3$) δ 0.69 (s, 3H), 0.84 (s, 3H), 0.85 (s, 3H), 2.08 (s, 3H, OCOC$H_3$), 2.14 (m, 1H, H-9), 2.59 (dd, 1H, $J$ = 19.2, 0.5, Hz, H-12β), 2.78 (m, 1H, $J$ = 6.2, 3.5, 1.1, 0.5 Hz, H-13), 3.12 (dd, 1H, $J$ = 19.2, 6.2 Hz, H-12α), 3.18 (m, 1H, $J$ = 3.5, 3.4, 2.2, 0.9 Hz, H-14), 5.14 (d, 1H, $J$ = 2.2 Hz, H-17), 5.21 (dd, 1H, $J$ = 2.1, 0.9 Hz, H-17′), 5.85 (dd, 1H, $J$ = 3.4, 1.1 Hz, H-15), 6.24 (s, 1H, H-16); $^{13}$C nmr ($CDCl_3$) [$^{13}$C-nmr assignments made by comparison of the data to that of known compounds (1,2,7)] δ 13.7 (q), 20.0 (t), 20.5 (2C, q and t), 21.0 (q), 24.9 (t), 32.5 (t, C-12), 33.1 (q, C-19), 33.3 (s, C-4), 38.1 (d, C-13), 39.6 (t), 41.4 (t), 43.3 (s, C-10), 46.9 (d, C-14), 56.0 (d), 58.8 (d), 100.6 (d, C-15), 101.2 (d, C-16), 116.3 (t, C-17), 140.3 (s, C-8), 166.8 (s, C-11), 169.4 (s, OCOMe); hrms (ei) $m/z$ [M]$^+$ 376.2261 ($C_{22}H_{32}O_5$ requires 376.2250); eims $m/z$ (rel. int.) [M]$^+$ 376 (4%), [M − Me]$^+$ 361 (3%), [M − HOAc]$^+$ 316 (43%), [M − Me − HOAc]$^+$ 301 (12%), [$C_{14}H_{24}$]$^+$ 192 (26%), [$C_{11}H_{18}$]$^+$ 150 (32%), [$C_{10}H_{17}$]$^+$ 137 (74%), [$C_9H_{15}$]$^+$ 123 (100%).

REDUCTION AND ACETYLATION OF NORRISOLIDE [**5**].—Norrisolide [**5**] (10.0 mg) was dissolved in dry $Et_2O$ (2 ml) and added to a suspension of $LiAlH_4$ (23.5 mg) in dry $Et_2O$ (2 ml). The reaction mixture was refluxed for 4 h, whereupon the reaction was quenched by addition of EtOAc (7 ml) and 0.5 M HCl (5 ml). The aqueous and organic layers were separated, and the aqueous layer was extracted with EtOAc (3 × 7 ml). The combined organic extracts were dried over $Na_2SO_4$ and filtered, and the solvent was removed to yield a clear oil (8.5 mg). $Ac_2O$ (0.5 ml) was added to a solution of this oil in pyridine (2 ml), and the reaction mixture was stirred at room temperature for 24 h. The solvents were removed in vacuo to yield

a crude product mixture (10.9 mg) which was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, column size 5.0 × 0.7 cm) using hexane-$Et_2O$ (3:2) as eluent to obtain the triacetate **15** (9.0 mg, 76% yield) as the major product.

TRIACETATE **15**.—Clear oil; $[\alpha]D$ +15.5° ($c$ = 0.38, $CHCl_3$); ir ($CHCl_3$) 1740, 1730, 1645, 1390, 1370, 1235, 1035 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 0.68 (s, 3H), 0.84 (s, 3H), 0.85 (s, 3H), 1.05 (m, 2H, $CH_2$-3), 1.23 (dd, 1H, $J$ = 12.8, 7.0 Hz, H-5), 1.42 (m, 3H, H-1, -2, -6), 1.65 (m, 6H, H-1′, -2′, -6′, -12, $CH_2$-7), 1.95 (m, 1H, H-12′), 2.03 (s, 3H, $OCOCH_3$), 2.04 (m, 1H, H-9), 2.04 (s, 3H, $OCOCH_3$), 2.05 (s, 3H, $OCOCH_3$), 2.12 (m, 1H, H-13), 2.39 (m, 1H, H-14), 4.08 (m, 3H, H-11, -15, -16), 4.18 (m, 3H, H-11′, -15′, -16′), 5.00 (s, 1H, H-17), 5.02 (s, 1H, H-17′); $^{13}C$ nmr ($CDCl_3$) δ 13.8 (q, C-20), 19.9 (t), 20.5 (2C, q and t), 20.9 (q), 21.0 (q, 2C), 25.8 (t), 26.9 (t), 33.2 (2C, q and s, C-19 and C-4), 34.8 (d, C-13), 39.3 (t), 41.5 (t), 43.6 (s, C-10), 44.8 (d, C-14), 58.1 (d), 58.7 (d), 62.6 (t), 64.1 (t), 65.3 (t), 113.0 (t, C-17), 147.0 (s, C-8), 170.9 (s, 2C, 2 × OCOMe), 171.0 (s, OCOMe); hrms (ei) $m/z$ $[M]^+$ 450.2968 ($C_{26}H_{42}O_6$ requires 450.2981); eims $m/z$ (rel. int.) $[M]^+$ 450 (4%), $[M-HOAc]^+$ 390 (44%), $[M-(HOAc)_2]^+$ 330 (19%), $[M-(HOAc)_3]^+$270 (24%), $[C_{14}H_{23}]^+$ 191 (20%), $[C_{11}H_{18}]^+$ 150 (100%), $[C_9H_{15}]^+$ 123 (64%).

REDUCTION AND ACETYLATION OF CHELONAPLYSIN A [**8**].—Chelonaplysin A [**8**] (6.6 mg) was dissolved in dry $Et_2O$ (1.5 ml) and added to a suspension of $LiAlH_4$ (9.0 mg) in dry $Et_2O$ (1 ml). The reaction mixture was refluxed for 4 h, whereupon the reaction was quenched by addition of EtOAc (7 ml) and 0.5 M HCl (5 ml). The aqueous and organic layers were separated, and the aqueous layer was extracted with EtOAc (3 × 7 ml). The combined organic extracts were dried over $Na_2SO_4$ and filtered, and the solvent was removed to yield a clear oil (3.8 mg). $Ac_2O$ (0.25 ml) was added to a solution of this oil in pyridine (1.5 ml), and the reaction mixture was stirred at room temperature for 16 h. The solvents were removed in vacuo to yield a crude product mixture (4.9 mg), which was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, column size 6.0 × 0.7 cm) using hexane-$Et_2O$ (3:2) as eluent to obtain the tetraacetate **12** (1.8 mg, 25% yield).

REDUCTION AND ACETYLATION OF CHELONAPLYSIN C [**10**].—Chelonaplysin C [**10**] (5.6 mg) was dissolved in dry $Et_2O$ (1.5 ml) and added to a suspension of $LiAlH_4$ (14.1 mg) in dry $Et_2O$ (1 ml). The reaction mixture was refluxed for 3 h, whereupon the reaction was quenched by addition of EtOAc (7 ml) and 0.5 M HCl (5 ml). The aqueous and organic layers were separated, and the aqueous layer was extracted with EtOAc (3 × 7 ml). The combined organic extracts were dried over $Na_2SO_4$ and filtered, and the solvent was removed to yield a clear oil (3.8 mg). $Ac_2O$ (0.25 ml) was added to a solution of this oil in pyridine (1.5 ml), and the reaction mixture was stirred at room temperature for 21 h. The solvents were removed in vacuo to yield a crude product mixture (4.7 mg), which was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, column size 5.5 × 0.7 cm) using hexane-$Et_2O$ (3:1) as eluent to obtain the triacetate **15** (4.9 mg, 73% yield) as the major product.

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