Swinholides and New Acetylenic Compounds from an Undescribed Species of *Theonella* Sponge

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Four new acetylenic compounds, **5**−**8**, along with four known ones, **1**−**4**, were isolated from a sponge, *Theonella* sp., collected in Chuuk Atoll, Federated States of Micronesia. Three unrelated swinholide-type compounds were also isolated. The structures of the new acetylenes were determined from spectral data and chemical degradation.

Marine sponges of the genus *Theonella* have proven to be a rich source of secondary metabolites with unique structures and intriguing biological activities.[1] Among these metabolites, macrolides[2] (e.g., the potent cytotoxin swinholide A[2a]) and peptides[3,4] (such as theonegramide,[4a] cupolamide,[4b] and oriamide[4c]) predominate. A recent investigation[5] of samples of *Theonella swinhoei* collected in Palau revealed that representatives of these two classes of metabolites, that is, swinholide A (a macrolide) and a cyclic peptide closely related to theonegramide, are produced by unicellular heterotrophic bacteria and filamentous bacteria, respectively, but not by blue-green algae, even though all three of these symbionts were present in the sponge. Our attention was drawn to an undescribed *Theonella* sponge, because the CH_2Cl_2-soluble extracts thereof exhibited cytotoxicity against several solid tumor cell lines. The sponge itself was morphologically very unusual in that it lacked the desma and surface triaene spicules typical of *Theonella* spp. The only morphological clue to the genus identity was the presence of tracts of strongyles and a dense crust of microrhabd spicules on the surface of the sponge. From the CH_2Cl_2-soluble fraction, we isolated swinholide A, preswinholide methyl and ethyl esters, and acetylenic metabolites **1**−**8**. Swinholide A was assumed to be the primary cause of the cytotoxicity. Quite a few acetylenic compounds have previously been isolated from marine sponges,[1] especially those of the genera *Petrosia*,[6] *Xestospongia*,[7] and *Pellina*.[8] In this paper, we report the isolation and structure elucidation of four new acetylenic compounds from a *Theonella* sp. To the best of our knowledge this is the first report of the isolation of acetylenic compounds from a *Theonella* sponge or other Lithistida sponges.

Specimens were extracted with MeOH and then MeOH−CH_2Cl_2 (1:1), and the combined extracts, after removal of solvents, were subjected to solvent partitioning[9] to give hexane, CH_2Cl_2, and *n*-BuOH fractions. The CH_2Cl_2 extract was cytotoxic and was fractionated by Si gel column chromatography, and fractions therefrom were further purified by repeated C_{18} reversed-phase HPLC to afford compounds **1**−**8** and the three swinholide-type compounds.

The known compounds swinholide A[2a] and preswinholide A methyl and ethyl esters[2e] were identified by comparison of their MS and ^{1}H NMR data with literature values. The MS and NMR data of compound **1** were essentially identical to those of pellynol A[8b] and melyne A.[10] Ozonolysis of **1** followed by oxidation with H_2O_2 and methylation of the resulting dicarboxylic acids with CH_2N_2 yielded dimethyl dodecandioate as a major component as evidenced by GC−MS analysis [*m*/*z* 227 $(M - OMe)^+$ for the major GC peak]. Hence, **1** was identified as melyne A.[10] The known acetylenes **2**−**4** were identified as melyne B,[10] pellynol C,[8b] and pellynol D,[8b] respectively, based on spectral data and oxidative degradation experiments as described for **1**.

Pellynol E (**5**) was obtained as a gum, $[\alpha]_D$ −8.3° (*c* 0.6, $CHCl_3$). The HRFABMS [obsd for $(M + Na)^+$ 507.3799; calcd 507.3814] in combination with NMR data confirmed a molecular formula of $C_{32}H_{52}O_3$. The ^{1}H and ^{13}C NMR spectra of **5** showed signals in good agreement with partial structure **a** [δ_H 5.91 (ddt, J = 15.5, 1.5, 7.0 Hz, H-28), 5.60

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(ddt, J = 15.5, 7.0, 1.5 Hz, H-29), 4.83 (br d, J = 7.0 Hz, H-30), 2.57 (d, J = 2.5 Hz, H-32) and δ_C 134.7 (C-28), 128.2 (C-29), 62.8 (C-30), 83.3 (C-31), 74.0 (C-32)] and substructure **b** [δ_H 4.35 (s, H2−1), 4.43 (t, J = 6.5 Hz, H-6); δ_C 51.4 (C-1), 77.4 (C-2), 69.8 (C-3), 68.8 (C-4), 80.5 (C-5), 62.8 (C-6)]. Spin systems were confirmed by $^1H-^1H$ COSY data. The combined small couplings of H-32 and -28 with H-30 resulted in broadening of the components of the larger coupling of the H-30 signal. Besides these, only resonances for a methylene chain were observed in the NMR spectra of **5**. Hence, the structure of pellynol E was assigned as **5**, a 16,17-dihydromelyne B.

Pellynol F (**6**), a minor component, was assigned the molecular formula $C_{33}H_{50}O_3$ based on NMR data and FABMS peaks at m/z 495 $[M + H]^+$ and 517 $[M + Na]^+$. The ^1H NMR spectrum of pellynol F (**6**) was similar to that of melyne A (**1**), except that the two-proton olefinic triplet at δ 5.33 in the spectrum of **1** was absent in that of pellynol F (**6**). Instead, a four-proton triplet at δ 2.14 (J = 6.5 Hz) was observed, and this was assigned to two methylene groups next to the central triple bond.[11] Consistent with the proposed central triple bond, the ^{13}C NMR spectrum of **6** showed an additional signal at δ 80.2 attributed to the medial sp carbons (C-17 and C-18).[11] An intense signal at δ 18.7 was assigned to C-16 and C-19, which are next to the triple bond.[11] From these data pellynol F was deduced to have a linear structure with the same terminal units **a** and **b** as in melyne A (**1**), and a triple bond flanked by methylene chains. The location of the central triple bond was determined by GC−MS analysis [m/z 227 $(M-OCH_3)^+$ for the major GC peak] of the mixture of products obtained from oxidative degradation (O_3; H_2O_2; CH_2N_2) of **6**. Therefore, pellynol F was determined to have structure **6**, a dehydro analogue of melyne A (**1**).

Pellynols G (**7**) and H (**8**) were the other two minor compounds isolated. However, pellynol H (**8**) could be an artifact of pellynol G (**7**), because it has been reported[12] that the trans ene−ol functionality in petrocortynes F−H was readily oxidized to an α,β-unsaturated ketone. In fact, **7** was totally converted to **8** upon standing a few days in $CDCl_3$. The molecular formula of pellynol G (**7**) was inferred to be $C_{33}H_{52}O_4$ based on its NMR data (Experimental Section) and the pseudomolecular ion peak at m/z 533 $[M + Na]^+$ in the MALDI−TOF MS of the oxidized product of pellynol G. By comparison of the ^1H and ^{13}C NMR data of pellynol G (**7**) with those of the known compounds, melynes A and B, partial structures **a** and **b** were easily recognized. In addition to substructures **a** and **b**, substructure **c** was also evident from the ^1H NMR data [δ 4.26 (dt, J = 8.0, 6.5 Hz, H-16), 5.35 (dd, J = 15.5, 8.0 Hz, H-17), and 5.76 (dt, J = 15.5, 6.5 Hz, H-18)], which were unambiguously assigned from $^1H-^1H$ COSY data. The associated carbons resonated at δ 87.1 (C-16), 128.5 (C-17), and 137.2 (C-18), as indicated by the HETCOR spectrum. The NMR data for substructure **c** were nearly identical to those of the same substructure in petrocortynes E−H.[12] The above data suggested for pellynol G a linear structure with the terminal units **a** and **b**, which contains the substructure **c** situated between methylene segments. The position and orientation of the trans ene−ol group was determined by GC−MS analysis of the methyl ester products obtained from oxidative degradation (O_3; H_2O_2; CH_2N_2). Two different samples of pellynol H were oxidized: (a) pellynol H from extraction of the sponge and (b) the product obtained from air oxidation of pellynol G (**7**). In each case dimethyl dodecandioate [(m/z 227 $(M-OCH_3)^+$] was obtained after the degradative procedure as a major component, as indicated by GC−MS analysis. This indicated that the double bond of substructure **c** and the $(CH_2)_{10}$ unit were directly connected. Therefore, the structure **7** was confirmed for pellynol G, and its oxidized product pellynol H was assigned structure **8**.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C_{18} column. IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument; UV spectra, on a Hewlett−Packard spectrophotometer. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3-mm $^1H/^{13}C$ switchable gradient microprobe (MDG-500−3) and a pulsed-field gradient driver; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer. MALDI−TOF mass spectra were taken on a PerSeptive Biosystems Voyages Elite instrument. GC−MS analysis was performed on a HP5985A GC−MS system. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter.

Animal Material. The sponge was collected from Nama Island, Chuuk Atoll, Micronesia, at a depth of 25 m in 1992. It forms a thick undulating encrustation with regularly spaced, raised oscules. The surface is smooth and wrinkled, compressible, and easily torn. Color in life was cream with a pinkish exterior. The skeleton consists of sinuous strongyles in vague tracts that are perpendicular to the surface of the sponge, and the interior and surface are packed with acanthose microrhabds. The general skeletal and tissue arrangement of the sponge is clearly of the genus *Theonella*, although desmas and surface triaenes are completely absent. The sponge is an undescribed species of *Theonella* (order Astrophorida, lithistid family Theonellidae). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1996:6:6:9), and the University of Oklahoma (7T92).

Extraction and Isolation. Freshly thawed specimens of the sponge (950 g wet wt; 140 g dry wt after extraction) were cut up and soaked in MeOH (2 × 1.6 L) followed by MeOH−CH_2Cl_2 (1:1) (2 × 1.6 L). All extracts were combined after removal of solvents *in vacuo* and subjected to solvent partitioning as described previously.[9] This gave, after evaporation of solvents *in vacuo*, hexane (2.68 g), CH_2Cl_2 (2.05 g), and *n*-BuOH (7.0 g) extracts. The CH_2Cl_2 extract was fractionated by flash chromatography over Si gel using increasing amounts of Me_2CO in CH_2Cl_2 as eluent (10% $Me_2CO-CH_2Cl_2$ to Me_2CO). Twelve fractions were collected. The sixth fraction was rechromatographed over C_{18} reversed-phase HPLC using 5% H_2O-CH_3CN as eluent to yield compounds **1** (10 mg), **2** (6 mg), **3** (2 mg), **4** (2 mg), **5** (8 mg), **7** (0.8 mg), and semi-pure compounds **6** (0.6 mg) and **8** (0.2 mg); the last two materials were each further purified by C_{18} reversed-phase HPLC using 10% H_2O-CH_3CN as the solvent system. Fraction 11, which was eluted with Me_2CO, contained minor amounts of swinholides and a mixture of sugars, and this was rechromatographed on Si gel column using 30% and then 35% $Me_2CO-CH_2Cl_2$ as eluents to give a mixture of swinholides. This mixture was further fractionated by reversed-phase C_{18} HPLC using 5% H_2O−MeOH as eluent to yield swinholide A and a mixture of pre-swinholide A methyl and ethyl esters, which in turn was resolved by reversed-phase HPLC employing 10% H_2O−MeOH as eluent.

Swinholide A: amorphous solid (7.0 mg), $[\alpha]_D$ + 18° (*c* 0.46, $CHCl_3$); FABMS m/z 1411 $[M + Na]^+$; NMR data identical with literature values.[2a]

Pre-swinholide A methyl ester: amorphous solid (4.5 mg), $[\alpha]_D$ −32.8° (*c* 0.22, $CHCl_3$); FABMS m/z 727 $[M + H]^+$, 749 $[M + Na]^+$; NMR data identical with literature values.[2e]

Pre-swinholide A ethyl ester: amorphous solid (4.0 mg), $[\alpha]_D$ −29.5° (*c* 0.18, $CHCl_3$); FABMS m/z 741 $[M + H]^+$, 763 $[M + Na]^+$; NMR data virtually identical to those of pre-swinholide A methyl ester except signals for an ethoxy group

[δ_C 60.3 (t), 14.3 (q); δ_H 4.20 (q, J = 7.0 Hz), 1.29 (t, J = 7.0 Hz)] instead of those for a methoxy group.

Pellynol E (5): $[\alpha]_D$ −8.3° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.91 (1H, ddt, J = 15.5, 1.5, 7.0 Hz, H-28), 5.60 (1H, ddt, J = 15.5, 7.0, 1.5 Hz, H-29), 4.83 (1H, dd, J = 7.0, 1.0 Hz, H-30), 4.43 (1H, t, J = 6.5 Hz, H-6), 4.35 (2H, s, H-1), 2.57 (1H, d, J = 2.5 Hz, H-32), 2.06 (2H, q, J = 7.0 Hz, H-27), 1.71 (2H, m, H-7), 1.43 (2H, m, H-8), 1.39 (2H, m, H-26), 1.26 (br s, remaining methylenic protons); ^{13}C NMR ($CDCl_3$) δ 134.7 (C-28), 128.2 (C-29), 83.3 (C-31), 80.5 (C-5), 77.4 (C-2), 74.0 (C-32), 69.8 (C-3), 68.8 (C-4), 62.8 (C-6 and C-30), 51.4 (C-1), 37.4 (C-7), 31.9 (C-27), 25.0 (C-8), 28.0−30.0 (other CH_2); FABMS m/z 507 $[M + Na]^+$; HRFABMS m/z 507.3799 (calcd for $C_{32}H_{52}O_3Na$ 507.3814).

Pellynol F (6): ^{1}H NMR ($CDCl_3$) δ 5.92 (1H, dt, J = 15.0, 7.0 Hz, H-29), 5.61 (1H, dd, J = 15.0, 6.5 Hz, H-30), 4.84 (1H, d, J = 6.5 Hz, H-31), 4.43 (1H, t, J = 7.0 Hz, H-6), 4.35 (2H, s, H-1), 2.57 (1H, d, J = 2.4 Hz, H-33), 2.14 (4H, t, J = 6.5 Hz, H-16 and H-19), 2.07 (2H, q, J = 7.0 Hz, H-28), 1.72 (2H, m, H-7), 1.46 (4H, m, H-15 and H-20), 1.42 (2H, m, H-8), 1.37 (2H, m, H-27), 1.27 (br s, remaining CH_2); ^{13}C NMR ($CDCl_3$) δ 134.6 (C-29), 128.3 (C-30), 83.3 (C-32), 80.5 (C-5), 80.2 (C-17 and C-18), 77.5 (C-2), 74.0 (C-33), 69.8 (C-3), 68.8 (C-4), 62.8 (C-6 and C-31), 51.4 (C-1), 37.4 (C-7), 31.9 (C-28), 25.0 (C-8), 18.7 (C-16 and C-19), 28−30 (remaining methylenic carbons); FABMS m/z 495 $[M + H]^+$, 517 $[M + Na]^+$.

Pellynol G (7): ^{1}H NMR ($CDCl_3$) δ 5.91 (1H, dt, J = 15.5, 6.5 Hz, H-29), 5.76 (1H, dt, J = 15.5, 6.5 Hz, H-18), 5.60 (1H, dd, J = 15.5, 6.5 Hz, H-30), 5.35 (1H, dd, J = 15.5, 8.0 Hz, H-17), 4.83 (1H, br d, J = 6.5 Hz, H-31), 4.42 (1H, t, J = 6.5 Hz, H-6), 4.34 (2H, s, H-1), 4.26 (1H, dt, J = 8.0, 6.5 Hz, H-16), 2.57 (1H, d, J = 1.5 Hz, H-33), 2.07 (2H, m, H-19), 2.05 (2H, m, H-28), 1.71 (2H, m, H-7), 1.62 (1H, m, H-15), 1.42 (3H, m, H-15 and H-8), 1.38 (4H, m, H-27 and H-20), 1.26 (br s, remaining methylenic protons); ^{13}C NMR ($CDCl_3$) (assignments by HETCOR experiment) δ 137.2 (C-18), 134.6 (C-29), 128.5 (C-17), 128.3 (C-30), 87.1 (C-16), 83.3 (C-32), 80.5 (C-5), 77.5 (C-2), 74.0 (C-33), 69.8 (C-3), 68.8 (C-4), 62.8 (C-6 and C-31), 51.4 (C-1), 37.4 (C-7), 32.3 (C-19), 31.9 (C-28), 25.3 (C-15), 24.9 (C-8), 28−30 (remaining methylenic carbons).

Pellynol H (8): ^{1}H NMR ($CDCl_3$) δ 6.82 (1H, dt, J = 15.5, 8.0 Hz, H-18), 6.09 (1H, d, J = 15.5 Hz, H-17), 5.91 (1H, dt, J = 15.5, 7.0 Hz, H-29), 5.61 (1H, dd, J = 15.5, 6.0 Hz, H-30), 4.84 (1H, d, J = 6.0 Hz, H-31), 4.43 (1H, t, J = 6.5 Hz, H-6), 4.35 (2H, s, H-1), 2.57 (1H, d, J = 2.0 Hz, H-33), 2.52 (2H, t, J = 7.1 Hz, H-15), 2.20 (2H, q, J = 7.0 Hz, H-19), 2.06 (2H, m, H-28), 1.70 (m, H-7), 1.59 (m, H-14), 1.46 (m, H-20), 1.38 (m, H-27), 1.26 (br s, remaining methylenic protons); MALDI−TOF MS m/z 533 $[M + Na]^+$.

Typical Procedure for Degradation of Acetylenic Compounds. Ozone was passed through a stirred solution of the acetylenic compound in CH_2Cl_2 at −78 °C until the solution turned pale blue. After removal of excess O_3 in the solution by bubbling in N_2, the reaction flask was removed from the cooling bath and allowed to reach room temperature. The reaction solution was evaporated by a stream of N_2. Glacial HOAc and 30% H_2O_2 were added to the reaction residue, and the resulting mixture was kept at room temperature overnight (14 h). The products were dried with a stream of N_2, dissolved in MeOH, and then treated with CH_2N_2 in Et_2O. After the solvent was removed by a stream of N_2, the products were subjected to GC−MS analysis.

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