Three New Acetylenes from the Palauan Sponge *Haliclona* sp.

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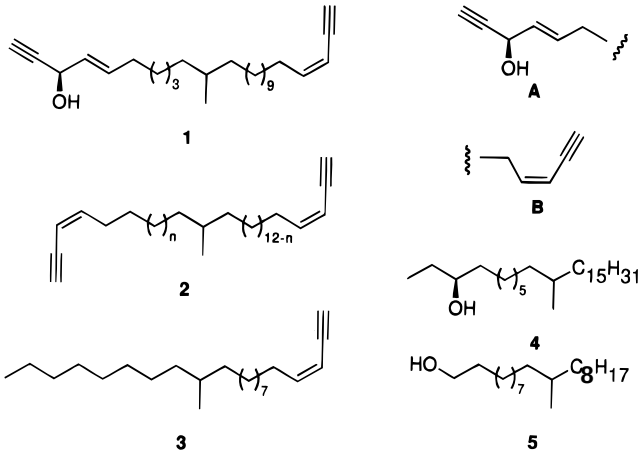
Three acetylenic metabolites **1**−**3** were isolated from the marine sponge *Haliclona* sp. from Palau, Western Caroline Islands. The structures were elucidated by interpretation of spectral data, and the absolute configuration of (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (**1**) was determined using the Mosher method.

Acetylenic and polyacetylenic compounds have previously been isolated from haplosclerid sponges of the genera *Cribrochalina*,[1] *Petrosia*,[2] *Siphonochalina*,[3] and *Xestospongia*.[4] In our pursuit of novel biologically active metabolites from marine invertebrates we have isolated three new acetylenes from a sponge *Haliclona* sp. collected in Palau. While the three new acetylenes (**1**−**3**) are simpler in structure than many of the polyacetylenic compounds reported previously,[1−4] they are unusual because they possess a methyl substituent on the methylene backbone.

The sponge *Haliclona* sp. was collected by hand using scuba (−18 m) from a fringing reef at Palau and was kept frozen until it was extracted with methanol. The methanol extract was reduced to 10% of its original volume and partitioned between dichloromethane and 15% methanol in water. The dichloromethane fraction was evaporated and partitioned between hexane and 10% aqueous methanol. The hexane fraction was evaporated and chromatographed on silica using a hexane:ethyl acetate gradient (95:5 → 0:100) to obtain (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (**1**, 171 mg, 0.085% dry weight). The least polar fraction was rechromatographed on silica using a hexane:ethyl acetate gradient (100:0 → 95:5) to obtain (3*Z*,23*Z*)-methylhexacosa-3,23-diene-1,25-diyne (**2**, 4 mg, 0.002% dry weight) and (3*Z*)-14-methyldocosa-3-en-1-yne (**3**, 4 mg, 0.002% dry weight).



(3*R*,4*E*,23*Z*)-3-Hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (**1**) was obtained as a colorless oil. The high resolution mass measurement indicated a molecular formula of $C_{27}H_{44}O$, and the mass spectrum also contained a strong $(M - OH)^+$ peak. The infrared spectrum contained bands at 3590 (OH), 3305 (C≡CH), and 2100 (C≡C) cm^{-1}. The ^{13}C NMR and ^1H NMR spectra, together with COSY, HMQC, and HMBC experiments, indicated the spin systems for two structural units (**A** and **B**). The assignment of the partial structure **A** is in complete accord with known (4*E*)-5-alkyl-3-hydroxypent-4-en-1-yne systems.[1−4] In the ^1H NMR spectrum, the acetylenic proton signal at δ 2.55 was coupled to the carbinol proton signal at 4.83 with a 2.5 Hz coupling constant. The latter signal was also coupled to an olefinic proton signal at δ 5.61 (dd, 1 H, J = 15.5, 6.5 Hz) that was in turn coupled to a second olefinic proton signal at 5.91 (dt, 1 H, J = 15.5, 6.5 Hz); the olefin was assigned the *E* geometry on the basis of the 15.5 Hz vicinal coupling constant. The olefinic proton signal at δ 5.91 was further coupled to an allylic methylene signal at 2.05 (br q, 2 H, J = 7 Hz). Partial structure **B** was defined by long-range coupling between the acetylenic proton signal at δ 3.06 (d, 1 H, J = 2.5 Hz) and the olefinic proton signal at 5.43 (br d, 1 H, J = 11 Hz), which was in turn coupled to an olefinic proton signal at 6.00 (dt, 1 H, J = 11, 7.5 Hz). The latter signal was also coupled to an allylic methylene signal at δ 2.31 (br q, 2 H, J = 7 Hz). The *Z* olefin geometry was assigned on the basis of the 11 Hz vicinal coupling constant. The data for the enyne system **B** are similar to those for reported terminal enynes isolated from *Petrosia* spp.[2,5,6] The ^1H NMR spectrum also contained a methyl signal at δ 0.82 (d, 3 H, J = 6 Hz), which showed long-range HMBC correlations to a methine carbon signal at δ 32.7 and to two equivalent methylene carbon signals at δ 37.1. These data established the presence of a methyl group on a methylene chain joining partial structures **A** and **B**.

The location of the methyl substituent was tentatively assigned on the basis of the fragmentation pattern of 11-methylhexacosanol (**4**), which was derived from alcohol **1** by hydrogenation over Adams' catalyst. The assignment of a methyl group at C-11 must be regarded as tentative because it is based on the relative intensity of the fragment ions: 111 ($C_8H_{15}^+$, 100), 125 ($C_9H_{17}^+$, 50), 139 ($C_{10}H_{19}^+$, 22), 153 ($C_{11}H_{21}^+$, 13), 167 ($C_{12}H_{23}^+$, 19), 181 ($C_{13}H_{25}^+$, 10). It is assumed that the fragment ions were derived primarily by cleavage of the alkyl chain of the carbonium ion formed by loss of a hydroxyl radical from alcohol **4**. The absolute configuration at C-3 was determined by Mosher's method.[6−8] The ^1H NMR spectra of the (*S*)- and (*R*)-MTPA esters of **1** were recorded and the $\delta_S - \delta_R$ values measured: these values

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(H-1 = −21, H-4 = +55, H-5 = +33, H-6 = +20 Hz) established the (*R*) configuration at C-3 of alcohol **1**. The absolute configuration at C-11 could not be determined.

(3*Z*,23*Z*)-Methylhexacosa-3,23-diene-1,25-diyne (**2**) was obtained as a colorless oil. The HRMS data indicated that the molecular formula was $C_{27}H_{44}$. The ^{1}H and ^{13}C NMR spectra were remarkably similar to those of alcohol **1** except that the signals associated with partial structure **A** were absent. Integration of the ^{1}H NMR spectrum established that acetylene **2** possessed two identical terminal enyne systems and a single methyl substitutent at an unknown location along the methylene chain joining the two terminal enyne units.

(3*Z*)-14-Methyldocosa-3-en-1-yne (**3**) was isolated as a colorless oil. The molecular formula $C_{23}H_{42}$ was determined by high-resolution mass measurement. Comparison of the ^{1}H and ^{13}C NMR spectra with those of acetylene **2** revealed that they were almost identical except that in the ^{1}H NMR spectrum of acetylene **3** there was an additional signal at δ 0.87 (t, 3 H, J = 7 Hz) that corresponded to the methyl signal in the ^{13}C NMR spectrum at δ 14.1. Integration of the ^{1}H NMR spectrum established that there was only one enyne group, which is attached to an alkyl chain having one methyl substituent on one of the interior carbon atoms. The location of the secondary methyl group was once again tentatively assigned on the basis of the mass spectrum fragmentation pattern of alcohol **5**, which was prepared from acetylene **3** by osmium tetraoxide/sodium periodate cleavage of the double bond and subsequent reduction of the resulting aldehyde with sodium borohydride. The location of the methyl group at C-11 was based on the relatively weak intensity of the m/z = 153 fragment ion: 111 ($C_8H_{15}^+$, 100), 125 ($C_9H_{17}^+$, 47), 139 ($C_{10}H_{19}^+$, 22), 153 ($C_{11}H_{21}^+$, 9), 167 ($C_{12}H_{23}^+$, 20), 181 ($C_{13}H_{25}^+$, 7).

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer using NaCl solution cells and UV spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. ^{1}H (500 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on Varian Unity 500 MHz and Bruker WP-200 SY spectrometers, respectively. All spectra are reported in $CDCl_3$ solutions with the chemicals shifts in ppm relative to chloroform (δ_H = 7.215, δ_C = 77.0) as internal standard. Low-resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer. High-resolution mass spectra were recorded on a VG ZAB mass spectrometer at the regional Mass Spectrometry Facility, UC Riverside.

Sponge Material. A specimen of the sponge *Haliclona* sp. (collection no. 95-122) was collected by hand using scuba (−18 m) from a fringing reef at Palau and was immediately frozen. A voucher specimen has been deposited in the Benthic Invertebrate Collection (no. P1160) at Scripps Institution of Oceanography.

Extraction and Purification. The frozen sponge was chopped into small pieces and extracted with methanol (2 × 500 mL) over a period of 2 days. The combined methanol extracts were concentrated under vacuum to give an aqueous suspension that was diluted with aqueous methanol (H_2O:MeOH, 85:15, 150 mL) and extracted with dichloromethane (2 × 200 mL). The combined dichloromethane extracts were evaporated under reduced pressure and partitioned between hexane and 10% aqueous methanol. The hexane layer was chromatographed on silica using a hexane:ethyl acetate gradient (95:5 → 0:100) to obtain (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (**1**). The least polar fraction was rechromatographed on silica using a hexane:ethyl acetate gradient (100:0 → 95:5) to obtain (3*Z*,23*Z*)-methylhexacosa-3,23-diene-1,25-diyne (**2**) and (3*Z*)-14-methyldocosa-3-en-1-yne (**3**).

(3*R*,4*E*,23*Z*)-3-Hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (1): oil; IR ($CHCl_3$) 3305, 2930, 2855, 2100, 1648 cm^{-1}; UV (MeOH) 227 (sh), 220, 214 (sh) nm; $[\alpha]_D$ = −5.6° (c = 0.93); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.00 (1 H, dt, J = 11, 7.5 Hz, H-23), 5.91 (1 H, dt, J = 15.5, 6.5 Hz, H-5), 5.61 (1 H, dd, J = 15.5, 6.5 Hz, H-4), 5.43 (1 H,br d, J = 11 Hz, H-24), 4.83 (1 H, br d, J = 6 Hz, H-3), 3.06 (1 H, br d, J = 2.5 Hz, H-26), 2.55 (1 H, d, J = 2.5 Hz, H-1), 2.31 (2 H, br q, J = 7 Hz, H-22), 2.05 (2 H, br q, J = 7 Hz, H-6), 1.38 (2 H, m, H-21), 1.25 (27 H, br s), 0.82 (3 H, d, J = 6.5 Hz, H-27); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.3 (C-23), 134.6 (C-5), 128.4 (C-4), 107.9 (C-24), 83.4 (C-2), 81.1 (C-26), 80.6 (C-25), 73.9 (C-1), 62.8 (C-3), 37.1(C-10, C-12), 32.7 (C-12), 31.9 (C-6), 30.3 (C-22), 30.0, 29.8, 29.7, 29.6, 29.5, 29.1, 28.8, 28.7, 27.1, 19.7 (C-27); CIMS (NH_3) m/z (int) $[M + NH_4]^+$ 402 (85), M^+ 384 (95), 367 (75), 135 (45), 95 (95), 81 (100); HRCIMS m/z 402.3738, calcd for $C_{27}H_{48}NO$ $[M + NH_4]^+$ 402.3736.

(3*Z*,23*Z*)-Methylhexacosa-3,23-diene-1,25-diyne (2): oil; IR ($CHCl_3$) 3306, 2930, 2855, 2100, 1648 cm^{-1}; UV (MeOH) 227 (sh), 220, 214 (sh) nm; $[\alpha]_D$ = −1° (c = 0.11); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.00 (2 H, dt, J = 10.5, 7.5 Hz, H-4, H-23), 5.43 (2 H, br d, J = 11.5 Hz, H-3, H-24), 3.06 (2 H, br d, J = 2 Hz, H-1, H-26), 2.31 (4 H, br q, J = 7 Hz, H-5, H-22), 1.39 (4 H, m), 1.28 − 1.25 (27 H, br m), 0.82 (3 H, d, J = 6.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.3 (C-23, C-4), 107.9 (C-24, C-3), 81.1 (C-26, C-1), 80.6 (C-25, C-2), 37.1, 32.8, 30.3, 30.0, 29.7, 29.6, 29.5, 29.2, 28.7, 27.1, 19.7; EIMS m/z (int) M^+ 368 (2.5), 171 (11), 131 (42), 117 (44), 81 (58), 67 (100); HRMS m/z 368.3451, calcd for $C_{27}H_{44}$ 368.3443.

(3*Z*)-14-Methyldocosa-3-en-1-yne (3): oil; IR ($CHCl_3$) 3305, 2930, 2855, 2100, 1648 cm^{-1}; UV (MeOH) 228 (sh), 220, 214 (sh) nm; $[\alpha]_D$ = −1° (c = 0.15); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.00 (1 H, dt, J = 11, 7.5 Hz, H-4), 5.43 (1 H, br d, J = 11.5 Hz, H-3), 3.06 (1 H, br d, J = 2 Hz, H-1), 2.32 (2 H, br q, J = 7 Hz, H-5), 1.39 (2 H, m, H-6), 1.28 −1.25 (29 H, br m), 0.87 (3 H, t, J = 7 Hz, H-22), 0.82 (3 H, d, J = 6.5 Hz, H-12′); ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.3 (C-4), 107.9 (C-3), 81.1 (C-1), 80.6 (C-2), 37.1 (C-10, C-12), 32.8 (C-11), 30.3 (C-5), 30.0, 29.7, 29.6, 29.4, 29.2, 27.1, 22.7, 19.7 (C-12'), 14.1 (C-22); EIMS m/z (int) M^+ 318 (13), 149 (35), 135 (49), 111 (65), 94 (100); HRCIMS m/z 318.3292, calcd for $C_{23}H_{42}$ 318.3287.

Mosher's Esters of (3*R*,4*E*,23*Z*)-3-Hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (1). A 1 M solution of dicyclohexycarbodiimide (15 μg, 1.5 equiv) in dichloromethane was added to a solution of the alcohol **1** (4 mg, 0.01 mmol) in dry dichloromethane (0.5 mL) containing 4-(dimethylamino)pyridine (1 mg) and either (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (3.5 mg, 1.5 equiv), and the solution was

stirred for 1 h at 25 °C. The reaction product was purified by flash chromatography on silica using 5−10% ethyl acetate in hexane to obtain the (*R*)- or (*S*)-MTPA ester (4.5 mg, 71%).

(*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetate of (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (1): oil; IR ($CHCl_3$) 3305, 2930, 2855, 2110. 2095, 1750 cm^{-1}; UV (MeOH) 264, 258, 253, 229 (sh) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 7.52 (2 H), 7.39 (3 H, m), 6.03 (1 H, br d, $J = 7$ Hz, H-3), 6.00 (1 H, dt, $J = 15$, 7 Hz, H-5), 6.00 (1 H, dt, $J = 11$, 7 Hz, H-23), 5.49 (1 H, dd, $J = 15$, 7 Hz, H-4), 5.43 (1 H, br d, $J = 11$ Hz, H-24), 3.59 (3 H, s, OMe), 3.06 (1 H, d, $J = 2$ Hz, H-26), 2.62 (1 H, d, $J = 2$ Hz, H-1), 2.31 (2 H, br q, $J = 7$ Hz, H-22), 2.04 (2 H, br q, $J = 7$ Hz, H-6), 0.82 (3 H, d, $J = 7$ Hz); HRCIMS *m/z* 618.4119, calcd for $C_{37}H_{55}NO_3F_3$ $[M + NH_4]^+$ 618.4134.

(*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetate of (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (1): oil; IR ($CHCl_3$) 3310, 2930, 2855, 2110. 2095, 1750 cm^{-1}; UV (MeOH) 265, 259, 256, 228 (sh) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 7.55 (2 H), 7.39 (3 H, m), 6.07 (1 H, dt, $J = 15$, 7 Hz, H-5), 6.01 (1 H, br d, $J = 7$ Hz, H-3), 6.00 (1 H, dt, $J = 11$, 7 Hz, H-23), 5.60 (1 H, dd, $J = 15$, 7 Hz, H-4), 5.43 (1 H, br d, $J = 11$ Hz, H-24), 3.55 (3 H, s, OMe), 3.06 (1 H, d, $J = 2$ Hz, H-26), 2.58 (1 H, d, $J = 2$ Hz, H-1), 2.31 (2 H, br q, $J = 7$ Hz, H-22), 2.08 (2 H, br q, $J = 7$ Hz, H-6), 0.82 (3 H, d, $J = 7$ Hz); HRCIMS *m/z* 618.4120, calcd for $C_{37}H_{55}NO_3F_3$ $[M + NH_4]^+$ 618.4134.

(3*R*)-11-Methylhexacosan-3-ol (4). A solution of 3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne (**1**, 1 mg) in methanol (3 mL) containing Adams' catalyst (0.2 mg) was stirred under an atmosphere of hydrogen for 24 h. The reaction mixture was filtered through celite to remove the catalyst, and the solvent was evaporated to obtain (3*R*)-11-methylhexacosan-3-ol (**4**): ^1H NMR (200 MHz, $CDCl_3$) δ 3.51 (1 H, m, H-3), 1.40 (2 H, m), 1.24 (43 H, br s), 0.93 (3 H, t, $J = 7$ Hz), 0.87 (3 H, t, $J = 7$ Hz), 0.82 (3 H, d, $J = 6$ Hz); EIMS *m/z* (int) $[M]^+$ 396 (1), $[M - H]^+$ 395 (4), $[M - H_2O]^+$ 378 (12), $[M - Et]^+$ 367 (63), $[M - EtCO]^+$ 339 (8), 181 ($C_{13}H_{25}^+$, 10), 167 ($C_{12}H_{23}^+$, 19), 153 ($C_{11}H_{21}^+$, 13), 139 ($C_{10}H_{19}^+$, 22), 125 ($C_9H_{17}^+$, 50), 111 ($C_8H_{15}^+$, 100).

11-Methylnonadecanol (5). A mixture of 14-methyldocosa-3-en-1-yne (**3**, 0.5 mg), potassium periodate (5 mg) in 2-methyl-2-propanol/water (1/1, 1 mL), and osmium tetraoxide (2 drops of 2.5% solution in 2-methyl-2-propanol) was stirred until thin layer chromatography revealed the absence of starting material. Sodium sulfite (10 mg) was added to the solution, which was stirred for 30 min. The mixture was diluted with ethyl acetate (40 mL), and the organic phase was washed with water (2 × 10 mL) and brine (10 mL) and then dried over sodium sulfate and the solvent evaporated. The residue was purified by flash chromatography with ethyl acetate:hexane (25:75) as eluent to obtain 11-methylnonadecanal: ^1H NMR (200 MHz, $CDCl_3$) δ 9.74 (1 H, t, $J = 2$ Hz), 2.41 (2 H, td, $J = 7$, 2 Hz), 1.60 (2 H, m), 1.24 (br, s), 0.87 (3 H, t, $J = 7$ Hz), 0.82 (3 H, d, $J = 6.5$ Hz); EIMS *m/z* (int) $[M]^+$ 296 (17), 278 (11), 111 (100).

Sodium borohydride (2 mg) was added to a stirred solution of 11-methylnonadecanal (0.2 mg) in methanol (0.5 mL). After 30 min, the solution was diluted with dichloromethane (20 mL) and washed with 2.5% aqueous ammonium chloride solution (4 mL). The organic layer was washed with brine (5 mL) and dried over sodium sulfate and the solvent evaporated under reduced pressure to obtain 11-methylnonadecanol (**5**): ^1H NMR (200 MHz, $CDCl_3$) 3.63 (2 H, t, $J = 7$ Hz), 1.24 (br s), 0.87 (3 H, t, $J = 7$ Hz), 0.82 (3 H, d, $J = 6.5$ Hz); EIMS *m/z* (int) $[M - H_2O]^+$ 280 (4), 181 ($C_{13}H_{25}^+$, 7), 167 ($C_{12}H_{23}^+$, 20), 153 ($C_{11}H_{21}^+$, 9), 139 ($C_{10}H_{19}^+$, 22), 125 ($C_9H_{17}^+$, 47), 111 ($C_8H_{15}^+$, 100).

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