Metabolites from the Sea Hare *Aplysia fasciata*

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Examination of the digestive and hermaphroditic glands' organic extract of the sea hare *Aplysia fasciata* yielded 16 metabolites, including eight sesquiterpenes, three diterpenes, and five C_{15}-acetogenins. Among them, three sesquiterpenes, 6-hydroxy-1-brasilene (**1**), epibrasilenol acetate (**2**), and 6-*epi*-β-snyderol (**3**), one acetogenin, (3*Z*,9*Z*)-7-chloro-6-hydroxy-12-oxo-pentadeca-3,9-dien-1-yne (**4**), and one diterpene, 16-acetoxy-15-bromo-7-hydroxy-9(11)-parguerene (**5**), are new natural products. The structure elucidation and the assignment of the relative configurations of the isolated natural products were established on the basis of extensive analyses of their spectroscopic data (NMR, MS, IR).

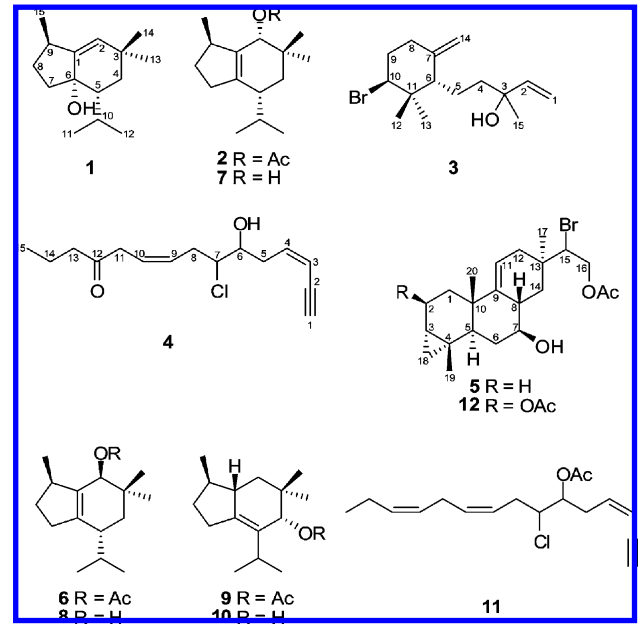
Sea hares are soft-bodied and slow-moving benthic marine animals that usually feed on a variety of marine algae. They have been proven to be a prolific source of unique bioactive compounds, which are generally contained in minute amounts and are often considered to be of dietary origin and/or to be produced by symbiotic microbes.[1,2] A possible role of the secondary metabolites acquired through their diet in the defense system of the sea hares has been proposed, although the fact that many of these compounds are found in the digestive glands of the animals—not an optimal location for defense—has caused this hypothesis to be questioned.[3] In fact, a number of the chemical weapons isolated so far were found in the mantle (external part) of the mollusks.[4−6]

Species of the genus *Aplysia* (Mollusca, Anaspidea) are widely distributed throughout the tropical and subtropical waters of the world and, like other sea hares, thrive on algae, usually on *Laurencia* species, acquiring and accumulating many algal metabolites in their digestive glands. Specimens of different *Aplysia* species collected from various locations have afforded a wide spectrum of new and known compounds, mainly sesquiterpenes, diterpenes, and acetogenins, frequently halogenated.[1,2]

Chromatographic separations of the digestive and hermaphroditic glands' organic extract of *Aplysia fasciata* led to the isolation of 16 metabolites, five of which are new natural products. Herein, we report the isolation and structure elucidation of the new compounds, which include three sesquiterpenes (**1**−**3**), one C_{15}-acetogenin (**4**), and one diterpene (**5**).

The freeze-dried digestive and hermaphroditic glands of the sea hare *A. fasciata*, collected in the Alfacs Bay, Spain, were exhaustively extracted with a mixture of CH_2Cl_2/MeOH, and the organic extract was subsequently subjected to a series of chromatographic separations to allow for the isolation of compounds **1**−**5** and several known metabolites.

Compound **1**, isolated as a colorless oil, displayed an ion peak at *m*/*z* 221.1909 (HRFABMS), corresponding to $C_{15}H_{25}O$ and consistent with $[M - H]^+$. The fragment ion at *m*/*z* 204 $[M - H_2O]^+$ in the mass spectrum, as well as the absorption band at 3485 cm^{-1} in the IR spectrum, indicated the presence of a hydroxy group. The ^{13}C NMR spectrum revealed 15 carbon signals, which corresponded to three quaternary carbon atoms, four methines, three methylenes, and five methyls, as determined from DEPT experiments. Among them, evident were two olefinic carbons (one methine and one quaternary resonating at δ 129.2 and 148.3, respectively), one



oxygenated quaternary carbon (δ 79.9), and a shielded tetrasubstituted carbon (δ 33.0). Since the carbon−carbon double bond accounted for one of the three degrees of unsaturation, the molecular structure of **1** was determined as bicyclic. The structural elements displayed in the ^1H NMR spectrum included five methyl groups, two on quaternary (δ 0.89 and 1.02) and three on methine (δ 0.96, 0.97, and 0.99) carbon atoms, and an olefinic methine of a trisubstituted double bond (δ 5.05). Two of the five methyls (singlets) were assigned to a *gem*-dimethyl group, as indicated by the HMBC correlations of C-3 with H_3-13 and H_3-14, while two of the remaining (doublets) were part of an isopropyl moiety, as revealed from the cross-peaks of H-10/H_3-11 and H-10/H_3-12 in the COSY spectrum, as well as the correlations of C-5 and C-10 with both H_3-11 and H_3-12 in the HMBC spectrum. The above information, in conjunction with further correlations provided by HSQC, HMBC, and COSY experiments, suggested a brasilane skeleton with a trisubstituted double bond and one oxygenated site.[7−9] The correlations of C-2 with H-9, H_3-13, and H_3-14 positioned the trisubstituted double bond between C-1 and C-2, whereas the correlations of C-6 with H-2, H_2-4, and H-10 placed the hydroxy group at C-6. The relative configuration of **1** was assigned on the basis of interactions observed in the NOESY spectrum. Specifically, the NOE enhancement of H-5/H_3-14 suggested an equatorial orientation for the isopropyl group and a

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pseudoaxial orientation for H_3-14 on the cyclohexene ring. Taking that into account, the only possible stereostructure that could justify the critical NOE correlation observed between H-7β and H_3-15 in the cyclopentane ring required a pseudoaxial orientation for both H_3-15 and the hydroxy group at C-6. The NOE enhancement of H-5/H-7β, even though partially overlapping with the H-5/H-10 cross-peak, further supported the proposed relative configuration of **1**.

Compound **2**, isolated as a colorless oil, displayed an ion peak at *m/z* 265.2195 (HRFABMS), corresponding to $C_{17}H_{29}O_2$ and consistent with $[M + H]^+$. The fragment ion at *m/z* 204 $[M - AcOH]^+$ in the mass spectrum, in conjunction with the absorption band at 1733 cm^{-1} in the IR spectrum, indicated the presence of an acetoxy group. The ^1H NMR spectrum of **2**, which included signals for five methyl groups (two on quaternary carbons resonating at δ 0.82 and 0.90 and three on methine carbons at δ 0.70, 0.91, and 0.97), an acetate methyl group (δ 2.01), and a deshielded oxygenated methine (δ 4.96), closely resembled that of brasilenol acetate (**6**). Analysis of the 2D NMR spectra (HSQC, HMBC, and COSY) of **2** suggested the same planar structure, thus indicating that **2** and **6** were stereoisomers. Proof of the latter hypothesis and identification of **2** was provided by acetylation of epibrasilenol (**7**) with Ac_2O in pyridine for 16 h at 70 C, which yielded epibrasilenol acetate, identical in all respects to the natural product **2**.

Compound **3** was isolated as a colorless oil. The ^{13}C NMR spectrum and DEPT experiments exhibited 15 signals, corresponding to three methyl, six methylene, three methine, and three quaternary carbon atoms. One quaternary, one methine, and two methylene carbons resonated in the sp^2 region of the ^{13}C NMR spectrum at δ 146.1, 144.9, 111.8, and 111.7, indicating the presence of a monosubstituted and a 1,1-disubstituted double bond in the molecule. An oxygenated quaternary carbon (δ 73.2) and a halogenated methine carbon (δ 63.3) were also evident. Identifiable in the ^1H NMR spectrum were three methyl groups on quaternary carbons (δ 1.01, 1.04, and 1.25), five olefinic protons of the monosubstituted (δ 5.04, 5.17, and 5.84) and the 1,1-disubstituted (δ 4.59 and 4.78) double bonds, and a halogenated methine (δ 4.31). The structural features observed in the ^1H and ^{13}C NMR spectra of **3** were very similar to those of β-snyderol.[10,11] Careful examination of the homonuclear and heteronuclear correlations exhibited in the HSQC, HMBC, and COSY spectra, as well as of the fragment ions in the mass spectrum, suggested the planar structure of β-snyderol. The relative configuration of metabolite **3** was assigned on the basis of NOE enhancements and coupling constant values of certain proton signals. Specifically, the fact that the methine at C-10 exhibited one large (11.2 Hz) and one medium (4.0 Hz) coupling constant with H_2-9 implied an axial orientation for H-10 and thus an equatorial orientation for the bromine atom, as in the case of β-snyderol.[11] However, the NOE correlations observed for H-6/H_3-12, H-9α/H-10, H-9β/H_3-12, and H-10/H_3-13 suggested that H-6 was equatorial. This was also supported by the lack of interaction between H-6 and H-10. The configuration at C-3 was not possible to be determined through spectroscopic analyses. Therefore, metabolite **3** was identified as the epimer of β-snyderol at C-6.

Compound **4**, isolated as a colorless oil, displayed a pseudomolecular ion peak at *m/z* 291.1140 (HRESIMS), corresponding to $C_{15}H_{21}ClNaO_2$ and consistent with $[M + Na]^+$. The mass spectrum exhibited pseudomolecular ions $[M + H]^+$ at *m/z* 269 and 271 (3:1) and fragment ions $[M - H_2O]^+$ and $[M - C_5H_6]^+$ at *m/z* 251 and 253 (2:0.7) and 203 and 205 (13:4), respectively, characteristic for the presence of one chlorine atom in the molecule. The ^{13}C NMR spectrum and DEPT experiments revealed 15 signals, corresponding to one methyl, five methylene, seven methine, and two quaternary carbon atoms. Among them, one carbonyl (δ 208.7), four methine sp^2 carbons (δ 111.1, 124.7, 128.1, and 140.3), two sp carbons of a triple bond (δ 77.2 and 82.4), a halogenated methine carbon (δ 66.5), and an oxygenated methine carbon (δ 71.9) were evident. The ^1H NMR spectrum included signals for one methyl group on a methylene carbon (δ 0.90), four olefinic methines (δ 5.59, 5.61, 5.73, and 6.08), two halogenated or oxygenated methines (δ 3.77 and 3.91), and one methine on a triple bond (δ 3.13). Since the carbonyl, the carbon−carbon triple bond, and the two carbon−carbon double bonds accounted for all five degrees of unsaturation, metabolite **4** was determined to be linear. The correlations between all geminal and vicinal protons observed in the COSY spectrum, as well as the HMBC correlations of C-12 with H_2-11, H_2-13, and H_2-14, assisted in establishing the structure of **4** unambiguously. The geometry of the Δ^3 and Δ^9 double bonds was assigned as *Z* on the basis of the measured coupling constants (using decoupling experiments) between H-3 and H-4 (J = 10.7 Hz) and H-9 and H-10 (J = 10.9 Hz). The relative configuration at C-6 and C-7 was not determined.

Compound **5** was isolated as a yellow oil. The combination of its ^{13}C NMR and HRFABMS data suggested the molecular formula $C_{22}H_{33}BrO_3$. The strong absorption bands at 3417 and 1734 cm^{-1} observed in the IR spectrum indicated the presence of a hydroxy group and an ester carbonyl, respectively. The ^{13}C NMR spectrum and DEPT experiments revealed 22 carbon signals, corresponding to four methyl, seven methylene, six methine, and five quaternary carbon atoms. Among them, one ester carbonyl (δ 170.7), two olefinic carbons (one methine and one quaternary resonating at δ 116.8 and 144.1, respectively), a halogenated methine carbon (δ 59.8), and two oxygenated carbons (one methylene and one methine resonating at δ 66.1 and 77.4, respectively) were evident. Since the carbonyl and the carbon−carbon double bond accounted for two of the six degrees of unsaturation, the molecular structure of **5** was determined as tetracyclic. The ^1H NMR spectrum confirmed the presence of three aliphatic methyl groups on quaternary carbons (δ 0.96, 0.97, and 1.05), one acetate methyl group (δ 2.09), one olefinic proton of a trisubstituted double bond (δ 5.33), and one trisubstituted cyclopropane ring (δ −0.05, 0.40, and 0.63). The correlations observed in the HSQC, HMBC, and COSY spectra of **5** suggested a parguerane skeleton with a trisubstituted double bond, one halogenated and two oxygenated carbons.[12] The heteronuclear correlations observed between H-11 and C-8, C-10, and C-13 positioned the trisubstituted double bond between C-9 and C-11. The acetoxy group was placed at C-16 on the basis of HMBC correlations of H_2-16 with C-13, C-17, and C-21, the hydroxy group was placed at C-7 due to the H_2-6 and H-8 interactions with C-7, and the correlations of C-13, C-16, and C-17 with H-15 positioned the bromine atom at C-15. The relative configuration of **5** was determined on the basis of NOE enhancements and coupling constants of certain proton signals. Specifically, the fact that the methine at C-7 appeared as a triplet of doublets with two large (10.4 Hz) and one medium (4.6 Hz) coupling constant with H_2-6 and H-8 suggested an axial orientation for H-7 and H-8 and an equatorial orientation for the hydroxy group. In addition, the NOE correlations observed for H-3/H_3-19, H-3/H_3-20, H-5/H-7, H-5/H-18b, H-7/H-14α, H-8/H_3-20, and H-14α/H_3-17 provided evidence that H-3, H-8, H_3-19, and H_3-20 were on one side of the molecule, while H-5, H-7, and H_3-17 were on the opposite side. The configuration at C-15 could not be determined by spectroscopic analyses.

The known compounds were identified, by comparison of their spectroscopic and physical characteristics with those reported in the literature, as brasilenol acetate (**6**),[7] epibrasilenol (**7**),[7] brasilenol (**8**),[7] 4-acetoxy-5-brasilene (**9**),[8,9] 4-hydroxy-5-brasilene (**10**),[8,9] (3*Z*,9*Z*,12*Z*)-6-acetoxy-7-chloro-pentadeca-3,9,12-trien-1-yne (**11**),[13,14] 15-bromo-2,16-diacetoxy-7-hydroxy-9(11)-parguerene (**12**),[15] (3*Z*)-venustinene,[16] (3*Z*)-13-*epi*-pinnatifidenyne,[17] (3*E*)-laurenyne,[18] and luzodiol,[19] previously isolated from species of the red alga *Laurencia* and/or the sea hare *Aplysia*, with the exception of metabolite **9**, which was described in the past as a semisynthetic compound and is reported for the first time as a natural product.

Careful analysis of the spectroscopic data of compound **11** led to the reassignment of a number of ^{1}H and ^{13}C NMR resonances.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 1 dm cell. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained on a Perkin-Elmer Paragon 500 spectrometer. NMR spectra were recorded on Bruker AC 200, Bruker DRX 400, and Varian 600 spectrometers. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. The 2D experiments (HSQC, HMBC, COSY, NOESY) were performed using standard Bruker or Varian pulse sequences. High-resolution mass spectra were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN. Low-resolution EI mass spectra were recorded on either a Hewlett-Packard 5973 mass spectrometer or a Thermo Electron Corporation DSQ mass spectrometer using a direct-exposure probe. Low-resolution CI mass spectra were recorded in the positive mode on a Thermo Electron Corporation DSQ mass spectrometer using a direct-exposure probe and methane as the CI reagent gas. Column chromatography was performed using Kieselgel 60 (Merck). HPLC separations were conducted using a CECIL 1100 Series liquid chromatography pump equipped with a GBC LC-1240 refractive index detector, using a Kromasil 100 SIL (MZ-Analysentechnik, 25 cm × 8 mm) column. TLC was performed using Kieselgel 60 F_{254} (Merck aluminum support plates), and spots were detected after spraying with 15% H_2SO_4 in MeOH reagent and heating at 100 °C for 1 min. The lyophilization was carried out in a Telstar CRYODOS freeze-drier.

Animal Material. Three specimens of *Aplysia fasciata* were collected in the Alfacs Bay, Delta de l'Ebre (Tarragona, Catalonia, Spain), at a depth of 1−1.5 m, in January 2008. A voucher specimen of the sea hare (AF210108CA) has been deposited at the Department of Animal Biology (Invertebrates), Faculty of Biology, University of Barcelona.

Extraction and Isolation. The animals were carefully dissected, and the mantles and the different internal organs were separately processed. The freeze-dried digestive and hermaphroditic glands were exhaustively extracted with CH_2Cl_2/MeOH (2:1) at room temperature. Evaporation of the solvent in vacuo afforded a dark brown, oily residue (3.02 g), which was subjected to vacuum column chromatography on silica gel, using cyclohexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as the mobile phase, to afford nine fractions (A−I). Fraction C (20% EtOAc in cyclohexane, 54.7 mg) was further fractionated by solid-phase extraction on normal-phase cartridges to yield three fractions (C1−C3). Fraction C1 (24.1 mg) was subjected repeatedly to normal-phase HPLC, using *n*-hexane/EtOAc (98:2) as eluant, to yield compounds **1** (1.1 mg), **2** (0.7 mg), **3** (1.0 mg), **6** (0.6 mg), **8** (2.2 mg), **9** (0.6 mg), **10** (0.6 mg), **11** (0.8 mg), (3*Z*)-venustinene (8.0 mg), (3*Z*)-13-*epi*-pinnatifidenyne (1.3 mg), and (3*E*)-laurenyne (0.6 mg). Fraction C2 (29.0 mg) was subjected repeatedly to normal-phase HPLC, using cyclohexane/EtOAc (95:5) as eluant, to afford compounds **3** (1.0 mg) and **7** (0.8 mg). Fraction F (50% EtOAc in cyclohexane, 226.1 mg) was further fractionated by solid-phase extraction on normal-phase cartridges to yield eight fractions (F1−F8). Fractions F3 (30.6 mg), F4 (36.9 mg), and F5 (20.5 mg) were subjected repeatedly to normal-phase HPLC, using cyclohexane/EtOAc (75:25) as eluant, to yield compounds **4** (3.3 mg), **5** (8.4 mg), and **12** (11.3 mg). Fraction G (60% EtOAc in cyclohexane, 102.3 mg) was further fractionated by solid-phase extraction on normal-phase cartridges to yield seven fractions (G1−G7). Fractions G3 (23.9 mg), G4 (37.9 mg), and G5 (15.4 mg) were subjected repeatedly to normal-phase HPLC, using cyclohexane/EtOAc (75:25) as eluant, to afford compounds **5** (2.0 mg), **12** (10.4 mg), and luzodiol (6.8 mg).

6-Hydroxy-1-brasilene (1): colorless oil; $[\alpha]^{20}_D$ −70 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244.0 (2.32) nm; IR (thin film) ν_{max} 3485, 2956, 1383, 1264 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.05 (1H, brs, H-2), 2.68 (1H, m, H-9), 2.12 (1H, m, H-8a), 1.97 (1H, m, H-10), 1.95 (1H, m, H-7β), 1.50 (1H, m, H-7α), 1.38 (1H, m, H-4a), 1.33 (1H, m, H-4b), 1.24 (1H, m, H-5), 1.18 (1H, m, H-8b), 1.02 (3H, s, H-13), 0.99 (3H, d, J = 6.8 Hz, H-15), 0.97 (3H, d, J = 6.8 Hz, H-12), 0.96 (3H, d, J = 6.9 Hz, H-11), 0.89 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 148.3 (C, C-1), 129.2 (CH, C-2), 79.9 (C, C-6), 46.3 (CH, C-5), 37.4 (CH_2, C-7), 34.8 (CH_2, C-4), 33.9 (CH, C-9), 33.0 (C, C-3), 31.4 (CH_3, C-13), 30.9 (CH_2, C-8), 28.1 (CH, C-10), 27.7 (CH_3, C-14), 23.6 (CH_3, C-12), 19.6 (CH_3, C-11), 19.2 (CH_3, C-15); EIMS 70 eV *m*/*z* (rel int %) 222 (4), 204 (27), 189 (100), 179 (4), 161 (24), 147 (22), 131 (24), 119 (35), 105 (44), 91 (27), 77 (13), 55 (10); HRFABMS *m*/*z* 221.1909 [M − H]$^+$ (calcd for $C_{15}H_{25}O$, 221.1905).

Epibrasilenol acetate (2): colorless oil; $[\alpha]^{20}_D$ +10 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 243.0 (1.92) nm; IR (thin film) ν_{max} 2928, 1733, 1378, 1261 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.96 (1H, brs, H-2), 2.60 (1H, m, H-9), 2.27 (2H, m, H-7a), 2.08 (1H, m, H-8a), 2.05 (1H, m, H-5), 2.01 (3H, s, H-17), 1.98 (1H, m, H-10), 1.59 (1H, m, H-7b), 1.41 (1H, m, H-4a), 1.38 (1H, m, H-8b), 1.09 (1H, m, H-4b), 0.97 (3H, d, J = 6.9 Hz, H-15), 0.91 (3H, d, J = 6.8 Hz, H-12), 0.90 (3H, s, H-14), 0.82 (3H, s, H-13), 0.70 (3H, d, J = 6.8 Hz, H-11); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 171.7 (C, C-16), 141.8 (C, C-6), 138.0 (C, C-1), 72.3 (CH, C-2), 40.2 (CH, C-5), 39.6 (CH, C-9), 34.4 (CH_2, C-7), 33.9 (C, C-3), 32.0 (CH_2, C-8), 30.6 (CH_2, C-4), 29.6 (CH, C-10), 27.6 (CH_3, C-14), 24.7 (CH_3, C-13), 21.8 (CH_3, C-17), 20.8 (CH_3, C-12), 19.7 (CH_3, C-15), 17.3 (CH_3, C-11); EIMS 70 eV *m*/*z* (rel int %) 204 (37), 189 (100), 161 (38), 145 (19), 131 (16), 119 (34), 105 (34), 91 (18), 77 (9), 55 (6), 43 (17); HRFABMS *m*/*z* 265.2195 [M + H]$^+$ (calcd for $C_{17}H_{29}O_2$, 265.2168).

6-*epi*-β-Snyderol (3): colorless oil; $[\alpha]^{20}_D$ −36 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 242.5 (2.75) nm; IR (thin film) ν_{max} 3431, 2966, 1457, 1263, 897 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.84 (1H, dd, J = 17.4, 10.7 Hz, H-2), 5.17 (1H, brd, J = 17.4 Hz, H-1a), 5.04 (1H, brd, J = 10.7 Hz, H-1b), 4.78 (1H, brs, H-14a), 4.59 (1H, brs, H-14b), 4.31 (1H, dd, J = 11.2, 4.0 Hz, H-10), 2.14 (1H, m, H-9α), 2.11 (2H, m, H-8), 1.98 (1H, m, H-9β), 1.95 (1H, m, H-6), 1.58 (1H, m, H-5a), 1.39 (1H, m, H-4a), 1.28 (1H, m, H-5b), 1.27 (1H, m, H-4b), 1.25 (3H, s, H-15), 1.04 (3H, s, H-13), 1.01 (3H, s, H-12); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 146.1 (C, C-7), 144.9 (CH, C-2), 111.8 (CH_2, C-1), 111.7 (CH_2, C-14), 73.2 (C, C-3), 63.3 (CH, C-10), 55.2 (CH, C-6), 40.5 (CH_2, C-4), 39.9 (C, C-11), 34.7 (CH_2, C-9), 31.7 (CH_2, C-8), 28.3 (CH_3, C-15), 27.5 (CH_3, C-13), 23.7 (CH_3, C-12), 20.2 (CH_2, C-5); EIMS 70 eV *m*/*z* (rel int %) 282:284 (4:4), 267:269 (12:12), 254:256 (7:7), 240:242 (5:5), 225:227 (3:3), 203 (53), 187 (42), 173 (24), 159 (32), 145:147 (32:32), 131 (40), 119:121 (58:59), 105 (73), 91 (100), 79 (80), 71 (66), 55 (39), 41 (74); HRFABMS *m*/*z* 221.1882 [M − Br]$^+$ (calcd for $C_{15}H_{25}O$, 221.1905).

(3*Z*,9*Z*)-7-Chloro-6-hydroxy-12-oxo-pentadeca-3,9-dien-1-yne (4): colorless oil; $[\alpha]^{20}_D$ −9.5 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 225.0 (3.18) nm; IR (thin film) ν_{max} 2932, 1716, 1265 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.08 (1H, dt, J = 10.7, 7.4 Hz, H-4), 5.73 (1H, m, H-10), 5.61 (1H, m, H-9), 5.59 (1H, m, H-3), 3.91 (1H, ddd, J = 7.7, 6.8, 3.2 Hz, H-7), 3.77 (1H, m, H-6), 3.21 (2H, d, J = 7.1 Hz, H-11), 3.13 (1H, d, J = 2.0 Hz, H-1), 2.69 (1H, m, H-5a), 2.66 (1H, m, H-8a), 2.60 (1H, m, H-5b), 2.52 (1H, m, H-8b), 2.41 (2H, t, J = 7.3 Hz, H-13), 1.58 (2H, m, H-14), 0.90 (3H, t, J = 7.4 Hz, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 208.7 (C, C-12), 140.3 (CH, C-4), 128.1 (CH, C-9), 124.7 (CH, C-10), 111.1 (CH, C-3), 82.4 (CH, C-1), 77.2 (C, C-2), 71.9 (CH, C-6), 66.5 (CH, C-7), 44.7 (CH_2, C-13), 41.5 (CH_2, C-11), 35.6 (CH_2, C-5), 33.2 (CH_2, C-8), 17.2 (CH_2, C-14), 13.7 (CH_3, C-15); PCIMS CH_4 *m*/*z* (rel. int. %) 269:271 (3:1), 251:253 (2:0.7), 233 (10), 203:205 (13:4), 191 (3), 165 (8), 147 (15), 129 (10), 121 (8), 105 (13), 95 (9), 71 (100), 55(3); HRESIMS *m*/*z* 291.1140 [M + Na]$^+$ (calcd for $C_{15}H_{21}ClNaO_2$, 291.1128).

16-Acetoxy-15-bromo-7-hydroxy-9(11)-parguerene (5): yellow oil; $[\alpha]^{20}_D$ −62.4 (*c* 0.50, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 248.5 (2.96) nm; IR (thin film) ν_{max} 3417, 2930, 1734, 1265, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.33 (1H, brd, J = 6.2 Hz, H-11), 4.55 (1H, m, H-16a), 4.27 (1H, m, H-15), 4.25 (1H, m, H-16b), 3.14 (1H, td, J = 10.4, 4.6 Hz, H-7), 2.42 (1H, m, H-12β), 2.33 (1H, m, H-8), 2.24 (1H, m, H-14β), 2.11 (1H, m, H-6α), 2.09 (3H, s, H-22), 1.96 (1H, m, H-2β), 1.80 (1H, m, H-12α), 1.79 (1H, m, H-2α), 1.60 (1H, m, H-6β), 1.59 (1H, m, H-1β), 1.38 (1H, dd, J = 13.1, 10.3 Hz, H-14α), 1.12 (1H, dd, J = 13.5, 3.4 Hz, H-5), 1.05 (3H, s, H-17), 0.97 (3H, s, H-19), 0.96 (3H, s, H-20), 0.85 (1H, dt, J = 12.9, 6.0 Hz, H-1α), 0.63 (1H, dt, J = 9.2, 6.2 Hz, H-3), 0.40 (1H, dd, J = 9.3, 4.1 Hz, H-18a), −0.05 (1H, dd, J = 5.6, 4.4 Hz, H-18b); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 170.7 (C, C-21), 144.1 (C, C-9), 116.8 (CH, C-11), 77.4 (CH, C-7), 66.1 (CH_2, C-16), 59.8 (CH, C-15), 46.4 (CH, C-5), 38.9 (CH_2, C-12), 38.8 (CH, C-8), 38.2 (CH_2, C-14), 37.0 (C, C-10), 35.5 (C, C-13), 35.1 (CH_2, C-6), 31.0 (CH_2, C-1), 24.3 (CH_3, C-17), 24.0 (CH_3, C-19), 21.3 (CH_2, C-18), 21.0 (CH_3, C-22), 19.3 (CH_2, C-2), 19.2 (CH, C-3), 17.8

(CH_3, C-20), 15.9 (C, C-4); PCIMS CH_4 *m*/*z* (rel int %) 407:409 (11:11), 347:349 (21:21), 326 (7), 313 (5), 285 (16), 267 (100), 251 (30), 241 (33), 225 (16), 211 (15), 197 (14), 185 (17), 171 (20), 145 (27), 131 (17), 109 (20), 95 (24), 81 (19), 61 (59); HRFABMS *m*/*z* 365.1505 $[M - OAc]^+$ (calcd for $C_{20}H_{30}BrO$, 365.1480).

(3*Z*,9*Z*,12*Z*)-6-Acetoxy-7-chloro-pentadeca-3,9,12-trien-1-yne (11): ^1H NMR ($CDCl_3$, 400 MHz) δ 5.93 (1H, dt, $J = 10.9$, 7.5 Hz, H-4), 5.58 (1H, dd, $J = 10.9$, 2.2 Hz, H-3), 5.52 (1H, m, H-10), 5.42 (1H, m, H-9), 5.39 (1H, m, H-13), 5.24 (1H, m, H-12), 5.15 (1H, ddd, $J = 7.5$, 5.4, 4.0 Hz, H-6), 3.94 (1H, ddd, $J = 8.2$, 5.4, 4.0 Hz, H-7), 3.13 (1H, d, $J = 2.2$ Hz, H-1), 2.77 (2H, m, H-11), 2.74 (2H, m, H-5), 2.53 (2H, m, H-8), 2.10 (3H, s, H-17), 2.04 (2H, m, H-14), 0.95 (3H, t, $J = 7.5$ Hz, H-15); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 170.3 (C, C-16), 138.9 (CH, C-4), 132.4 (CH, C-10), 131.7 (CH, C-13), 126.4 (CH, C-12), 124.3 (CH, C-9), 111.7 (CH, C-3), 82.7 (CH, C-1), 77.2 (C, C-2), 73.4 (CH, C-6), 62.5 (CH, C-7), 32.4 (CH_2, C-5), 32.2 (CH_2, C-8), 25.7 (CH_2, C-11), 20.9 (CH_2, C-14), 20.6 (CH_3, C-17), 14.2 (CH_3, C-15).

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Supporting Information Available: ^1H NMR, ^{13}C NMR, and MS spectra of the new compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Blunt, J. W.; Copp, B. R.; Hu, W. P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2009**, *26*, 170–244, and earlier reviews in this series.
(2) MarinLit Database, Department of Chemistry, University of Canterbury: http://www.chem.canterbury.ac.nz/marinlit/marinlit.shtml.
(3) Pennings, S. C.; Paul, V. J. *Mar. Biol.* **1993**, *117*, 535–546.
(4) Ortega, M. J.; Zubía, E.; Salvá, J. *J. Nat. Prod.* **1997**, *60*, 488–489.
(5) Spinella, A.; Gavagnin, M.; Crispino, A.; Cimino, G.; Martínez, E.; Ortea, J.; Sodano, G. *J. Nat. Prod.* **1992**, *55*, 989–993.
(6) Miyamoto, T.; Higuchi, R.; Komori, T.; Fujioka, T.; Mihoshi, K. *Tetrahedron Lett.* **1986**, *27*, 1153–1156.
(7) Stallard, M. O.; Fenical, W.; Kittredge, J. S. *Tetrahedron* **1978**, *34*, 2077–2081.
(8) Amico, V.; Caccamese, S.; Neri, P.; Russo, G.; Foti, M. *Phytochemistry* **1991**, *30*, 1921–1927.
(9) Wright, A. D.; König, G. M.; Sticher, O. *J. Nat. Prod.* **1991**, *54*, 1025–1033.
(10) Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1976**, *17*, 41–44.
(11) Topcu, G.; Aydogmus, Z.; Imre, S.; Gören, A. C.; Pezzuto, J. M.; Clement, J. A.; Kingston, D. G. I. *J. Nat. Prod.* **2003**, *66*, 1505–1508.
(12) Schmitz, F. J.; Michaud, D. P.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 6415–6423.
(13) González, A. G.; Martín, J. D.; Martín, V. S.; Norte, M.; Pérez, R.; Ruano, J. Z.; Drexler, S. A.; Clardy, J. *Tetrahedron* **1982**, *38*, 1009–1014.
(14) Norte, M.; González, A. G.; Cataldo, F.; Rodríguez, M. L.; Brito, I. *Tetrahedron* **1991**, *47*, 9411–9418.
(15) Higgs, M. D.; Faulkner, D. J. *Phytochemistry* **1982**, *21*, 789–791.
(16) Suzuki, M.; Kurosawa, E.; Furusaki, A.; Matsumoto, T. *Chem. Lett.* **1983**, 779–782.
(17) San-Martin, A.; Darias, J.; Soto, H.; Contreras, C.; Herrera, J. S.; Rovirosa, J. *Nat. Prod. Lett.* **1997**, *10*, 303–311.
(18) Falsaw, C. P.; King, T. J.; Imre, S.; Islimyeli, S.; Thomson, R. H. *Tetrahedron Lett.* **1980**, *21*, 4951–4954.
(19) Kuniyoshi, M.; Wahome, P. G.; Miono, T.; Hashimoto, T.; Yokoyama, M.; Shrestha, K. L.; Higa, T. *J. Nat. Prod.* **2005**, *68*, 1314–1317.

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