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SEVEN NEW METABOLITES FROM THE MARINE RED ALGA *LAURENCIA MAJUSCULA*

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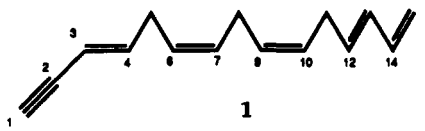
ABSTRACT.—From the marine red alga *Laurencia majuscula* seven new natural products, six C_{15} acetogenins **1–6** and one sesquiterpene **7**, have been isolated and characterized. Three of these compounds (**3–5**) possess the rare 2,5-dioxabicyclo[2.2.1]heptane ring system. For the previously reported sesquiterpene **8**, complete ^{1}H- and ^{13}C-nmr data are reported for the first time.

Algae of the genus *Laurencia* are prolific producers of many and varied natural products, particularly brominated and chlorinated compounds (1,2). A further interesting aspect of the chemistry of this genus is the chemical variation that can occur within a single species depending on place and time of collection. The Great Barrier Reef, Queensland, Australia, a rich resource of *Laurencia* species, is an ideal place to study such variation. Because no chemical studies had been performed on *Laurencia majuscula* (Harvey) Lucas (Rhodomelaceae) collected from a deep water coral reef, a study of such a sample was embarked upon to establish if the already noted chemical variation within this species (3,4) is more wide-spread.

RESULTS

The marine red alga *L. majuscula* was collected from Holmes Reef, a deep water coral reef, Queensland, Australia. Freeze-dried algal tissue was extracted with CH_2Cl_2 to yield a green gum. Cc of the material over Si gel followed by hplc separations, as described in the Experimental section, afforded eight pure compounds.

Compound **1**, a clear mobile oil, analyzed for $C_{15}H_{18}$ by ms. Of the six degrees of unsaturation implied by the molecular formula of **1**, all were occupied by either sp^2 or sp hybridized carbons; **1** was thus an acyclic molecule containing five carbon-carbon double bonds and a single carbon-carbon triple bond. From the ^{1}H-^{1}H and the ^{1}H-^{13}C 2D nmr COSY spectra of **1** it was possible to follow a continuous chain of coupling from C-1 through to C-15. Thus, H-1 showed coupling to both H-3 (J=2.3 Hz) and H-4 (J=0.6 Hz), which inter-coupled and had a trans relationship to each other (J=16.0



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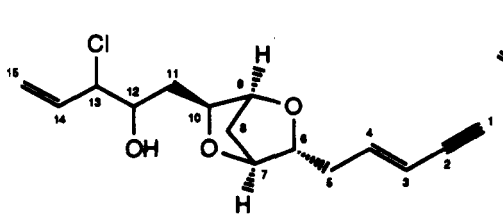

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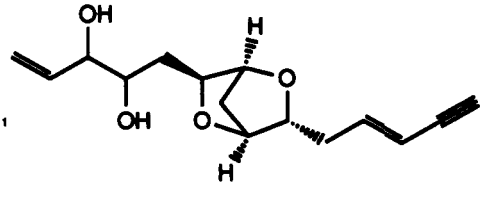
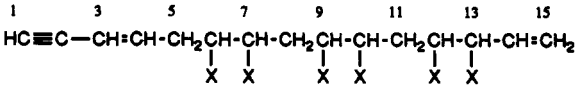


4

Hz). H-4 further coupled to both of the protons at C-5, which both had a vicinal coupling to H-6. This latter proton also coupled to H-7 and had a cis relationship to it (J=10.7 Hz). H-7 in turn coupled to both methylene protons at C-8, which inter-coupled and further coupled to H-9, which in turn coupled to H-10, H-9 and H-10 being cisoid (J<10 Hz). Further, H-10 coupled to the two remaining aliphatic protons at C-11, both of which coupled to H-12, which also coupled to H-13 and was trans to it (J=15.1 Hz). H-13 further coupled to H-14, which coupled to both of the *exo*-methylene protons at C-15. Compound **1** is (3*E*,6*Z*,9*Z*,12*E*)-pentadeca-3,6,9,12,14-pentaen-1-yne.

Compound **2**, by ms, was found to have the molecular formula $C_{15}H_{20}$. Comparison of the spectroscopic data of **2** with those of **1** revealed that **1** and **2** were identical in the regions C-1 to C-13. In **2** the $\Delta^{14,15}$ double bond of **1** was fully saturated, giving rise to a ^{1}H-nmr resonance for a methyl group, δ 1.07 (t,J=7.5 Hz), and for an allylic methylene function, δ 2.10 (br dq,J=7.5, 7.5 Hz), in the spectrum of **2**. The *E* configuration of the $\Delta^{12,13}$ double bond was deduced by considering the down-field shift of the C-14 carbon signal (25.9 ppm) compared to the corresponding ^{13}C-nmr shift of C-14 (20.6 ppm) in *trans*-laurencenyne (5). Compound **2** is (3*E*,6*Z*,9*Z*,12*E*)-pentadeca-3,6,9,12-tetraen-1-yne.

Compound **3**, by ms and ^{13}C-nmr spectroscopy, was found to have the molecular formula $C_{15}H_{19}O_3Cl$. Of the six degrees of unsaturation implied by the molecular formula of **3**, four were occupied in two carbon-carbon double bonds [111.3 (d), 141.9 (d), 134.0 (d), 119.8 (t) ppm] and a single carbon-carbon triple bond [76.4 (d), 82.1 (s) ppm]; **3** was thus bicyclic. From the ^{1}H-^{1}H and the ^{1}H-^{13}C (J=136 Hz) 2D nmr COSY spectra of **3**, it was possible to construct a continuous chain of carbon atoms from C-1 through to C-15 as shown in the following partial structure.



From the ir and ms data it was apparent that **3** must contain a secondary alcohol, a secondary chloro, and two ether functions. The ^{13}C-nmr data indicated the chloro function to reside at C-13 [67.0 (d) ppm], and the hydroxyl to be at C-12 [71.3 (d) ppm], leaving C-6, C-7, C-9, and C-10 as the sites where ether linkages will form. Possible linkages, for the two ethers, are between C-6 and C-7, C-9 and C-10; C-6 and C-9, C-7 and C-10; or C-6 and C-10, C-7 and C-9. The first possibility was quickly eliminated because the ^{13}C-nmr resonances for carbons belonging to epoxides would occur at much higher field (ca. 65 ppm). Of the two remaining possibilities, the one giving rise to two five-membered rings is clearly favored as previously discussed by Schulte *et al.* (6).

With the basic skeleton of **3** established, the stereochemistry at six chiral centers and the $\Delta^{3,4}$ double bond required resolution. The double bond was assigned the *E* configuration on the basis of the $J_{3,4}$ proton-proton coupling constant of 16.1 Hz. The stereochemistry at centers C-6, C-7, C-9, and C-10 was proposed from the results of a 2D NOESY experiment made with **3**, the diagnostic nOe's being observed between H-6 and H-8 and between H-5 and H-10. The configuration proposed for C-6 in **3** is in contrast to those reported by Schulte *et al.* (6) for *E* and *Z*- ocellenyne. Compound **3** is (3*E*,6*R**,7*R**,9*R**,10*S**)-6,9:7,10-bisepoxy-13-chloro-12-hydroxypentadeca-3,14-dien-1-yne.

Compound **4**, the most polar metabolite isolated, had the molecular formula $C_{15}H_{20}O_4$ as deduced by ms. From its ^{1}H- and ^{13}C-nmr data (see Experimental and Table 1) it was evident that **4** was the 13-hydroxyl derivative of **3**. Stereochemically **3** and **4**

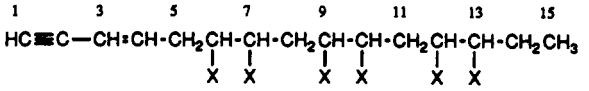
TABLE 1. ^{13}C-nmr Data ($CDCl_3$, 75.5 MHz) for Compounds **3**, **4**, and **5**.

Carbon	Compound		
	3	**4**	**5**
C-1	76.4 (d)	76.4 (d)	77.1 (d)
C-2	82.1 (s)	not obsvd	82.1 (s)
C-3	111.3 (d)	111.3 (d)	111.4 (d)
C-4	141.9 (d)	141.9 (d)	141.8 (d)
C-5	34.5 (t)	34.5 (t)	34.5 (t)
C-6	81.6 (d)	81.6 (d)	81.6 (d)
C-7	80.2 (d)	80.3 (d)	80.1 (d)
C-8	34.8 (t)	34.8(t)	34.9 (t)
C-9	77.0 (d)	76.9 (d)	76.4 (d)
C-10	81.7 (d)	82.2 (d)	81.9 (d)
C-11	36.7 (t)	35.6 (t)	39.7 (t)
C-12	71.3 (d)	70.9 (d)	68.8 (d)
C-13	67.0 (d)	76.2 (d)	136.9 (d)
C-14	134.0 (d)	136.3 (d)	126.3 (d)
C-15	119.8 (t)	117.9 (t)	44.2 (t)

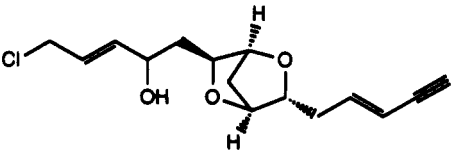
were found to be iso-structural. Compound **4** is (3*E*,6*R**,7*R**,9*R**,10*S**)-6,9:7,10-bisepoxy-12,13-dihydroxypentadeca-3,14-dien-1-yne.

Compound **5** analyzed for $C_{15}H_{19}O_3Cl$ by ms and was thus an isomer of **3**. Direct comparison of ^{1}H- and ^{13}C-nmr data (see Experimental and Table 1) revealed the two to be structurally identical from C-1 through to C-12. The C-13 to C-15 fragment in **5**, however, contained an endo-double bond and a terminal chloromethyl group, in contrast to the exo-double bond and *endo*-chloromethylene groups of **3**. The $\Delta^{13,14}$ double bond had the *E* configuration based on $J_{13,14}$ being 15.5 Hz. The results of a 2D NOESY measurement made with **5** confirmed it to have stereochemistry identical to that of **3** at C-6, C-7, C-9, and C-10. Compound **5** is (3*E*,6*R**,7*R**,9*R**,10*S**,13*E*)-6,9:7,10-bisepoxy-15-chloro-12-hydroxypentadeca-3,13-dien-1-yne.

Compound **6**, by ms, was found to have the molecular formula $C_{15}H_{21}O_3Cl$. From its uv, ir, ms, and nmr data it was evident that the functionalities within **6** were a secondary alcohol, a secondary chloro function, two ethers, and a terminal enyne moiety; the molecule was thus bicyclic. From the results of ^{1}H-^{1}H and ^{1}H-^{13}C (J=136 Hz) 2D nmr COSY measurements made with **6** it was possible to construct a continuous chain of carbon atoms from C-1 through to C-15 as shown below.



From these measurements it was also evident that the OH function must reside at C-12 [71.0 (d) ppm]. Further, the ^{13}C-nmr data indicated the secondary chloro function to be at C-7 [58.2 (d) ppm]. The positions of the two ether linkages remained to be assigned. They could occur between C-6 and C-9, C-10 and C-13; C-6 and C-10, C-9 and C-13; or between C-6 and C-13, C-9 and C-10. The latter case was not further considered as a possibility since the observed carbon shifts were at too low field for epoxides (ca. 65 ppm). Of the two remaining possibilites the one that generated the 1,5-dioxadecalin ring system was selected as the more likely based on data comparisons made with elatenyne (7) and (3*E*)-dactomelyne (8). Stereochemically, **6** proved to have the



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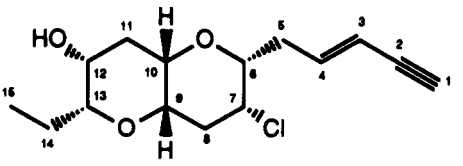


6

same relative stereochemistry at all centers as elatenyne, based on ^{1}H-^{1}H coupling information (Figure 1) and the results of a 2D NOESY measurement made with **6**. The $\Delta^{3,4}$ double bond was assigned the *E* configuration based on the $J_{3,4}$=15.9 Hz. Compound **6** is (3*E*,6*R**,7*R**,9*S**,10*S**,12*R**,13*R**)-6,10:9,13-bisepoxy-7-chloro-12-hydroxypentadeca-3-en-1-yne.

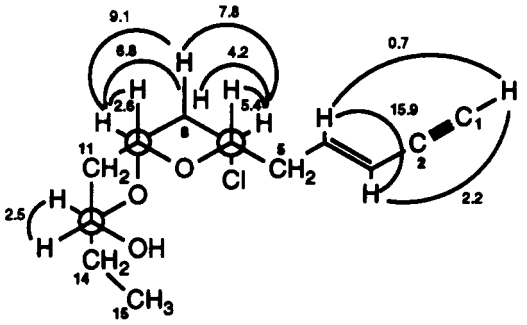
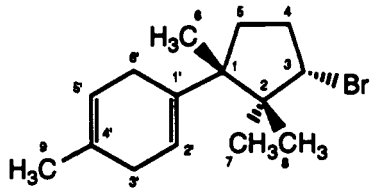
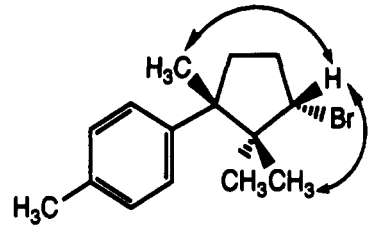


FIGURE 1. Newman projection of **6**. Coupling constants are given in Hz.

Compound **7** was a sesquiterpene with molecular formula $C_{15}H_{23}Br$ as determined by ms. From the ^{13}C-nmr spectrum of **7**, four resonances for sp^2 hybridized carbons could be observed, arising from two carbon-carbon double bonds; **7** was thus bicyclic. The results of ^{1}H-^{1}H and ^{1}H-^{13}C (J=136 Hz) 2D nmr COSY measurements enabled two distinct ^{1}H-^{1}H spin systems to be discerned. Thus, the proton with resonance at δ 4.30 (H-3) coupled to the two C-4 methylene protons (δ 2.38, 2.04), which were geminally coupled and further coupled to the protons at C-5 (δ 1.40, 2.34), completing the first spin system. The second system could be traced from the olefinic proton H-2′ (δ 5.55) to the protons at C-3′ (δ 2.64), and from there to the vinyl methyl group, C-9 (δ 1.67). From this methyl group coupling to H-5′ was apparent, as was coupling between H-5′ (δ 5.41) and both protons at H-6′ (δ 2.70), completing the second of the two proton spin systems. After this analysis three tertiary methyl groups and three quaternary carbon atoms required positioning. Of the three quaternary carbons one was associated with the $\Delta^{1',2'}$ double bond, leaving two. Since all methyl groups were tertiary and not allylic, two had to be involved in the *gem*-dimethyl constellation (C-2, C-7, C-8), with the third left to reside at C-1. At this point in the structural analysis it was apparent that **7** was an α-isobromocuparene [**8**] derivative (9). The difference between **7** and an α-isobromocuparene was the lack of aromaticity in **7**; in **7** the six-membered ring was present as a 1,4-cyclohexadiene moiety. The stereochemistry shown for **7** was deduced from the results of a 2D NOESY measurement made with **7**. Refluxing **7** in toluene containing a catalytic amount of palladium charcoal gave a quantitative yield of **8**. Compound **7** is (1*R**,3*S**)-3-bromo-1,2,2-trimethyl-1-(4′-methylcyclohexa-1′,4′- dien)cyclopentane.



7



8 Arrows indicate nOe's

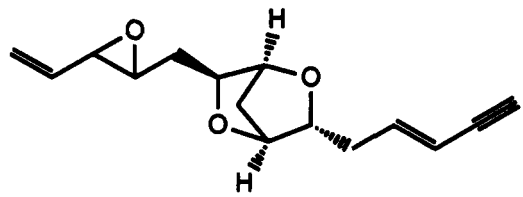
Compound **8** was isolated from the same vlc fraction as **7** and its structure confirmed to be identical with that of α-isobromocuparene [**8**]. A complete set of ^{1}H- and ^{13}C-nmr data is reported for **8** (see Experimental).

DISCUSSION

Previous investigations of *L. majuscula* from the Great Barrier Reef (3), the Japanese (2,11) and Italian coasts (12) led to the isolation of only chamigrene derivatives and an unusual triquinane-based sesquiterpene (3). The current study on *L. majuscula* from a deep water coral reef revealed that C_{15} acetogenins **1–6** are also present in this plant species.

Compounds **3–5** possess the rare 2,5-dioxabicyclo[2.2.1]heptane ring system, which to date has only been encountered in two metabolites isolated from the sea hare *Aplysia oculifera* (6). Possible biosynthetic precursors for these unusual cyclic ethers are the acetylenic polyenes **1** and **2**. Acyclic C_{15} acetogenins are already discussed in the literature as plausible precursors of various C_{15} ethers (13). The allylic epoxide **9** is proposed as a reasonable precursor of compounds **3–5**.

This investigation further underlines the fact that algae of the genus *Laurencia* contain many new and rare natural products, with the secondary metabolite content showing spatial, and possibly temporal, variation.



9

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The experimental procedures were as described by König *et al.* (10).

PLANT MATERIAL.—The algal material was collected in August 1989 from Holmes Reef, latitude 147° 52′ E, longitude 16° 30′ S, Queensland, Australia. Plants growing at 10–20 m depth were collected, deep frozen, and on return to the laboratory, freeze-dried. A voucher specimen is deposited at the James Cook University Department of Botany herbarium, Townsville, Australia (Voucher number JCT A7675).

EXTRACTION AND ISOLATION.—The dry algal tissue (67.3 g) was exhaustively extracted with 1 liter of CH_2Cl_2 and then with an equivalent volume of MeOH to afford 724 mg (1%) of CH_2Cl_2-soluble material. Vacuum liquid chromatography (vlc) (3) of this material over Si gel using hexane with increasing proportions of EtOAc as eluent afforded 13 fractions, each of approximately 80 ml. Tlc and ^{1}H-nmr investigation of these fractions indicated fractions 1–5 and 9–13 to be of further interest.

Hplc separation of fractions 1 and 2, on normal phase silica with hexane as eluent yielded the two C_{15} hydrocarbons, **1** and **2**, as well as two sesquiterpenes, the known compound **8** and the new natural product **7**.

Compound **1**.—(3*E*,6*Z*,9*Z*,12*E*)-Pentadeca-3,6,9,12,14-pentaen-1-yne [**1**] was obtained as an oil (5.2 mg, 0.007%): ir ν max (film) 3300, 3020, 2930, 2110, 1010, 955, 900 cm^{-1}; uv λ max (EtOH) 225 nm (ϵ 21,000); ^1H nmr (C_6D_6, 300 MHz) δ 2.65 (br dd, J=6.4, 6.7 Hz, 2H, H-5), 2.71 (dd, J=0.6, 2.3 Hz, 1H, H-1), 2.72 (br dd, J=6.2, 6.2 Hz, 2H, H-8), 2.81 (br dd, J=6.6, 6.7 Hz, 2H, H-11), 5.08 (br d, J=10.3 Hz, 1H, H-15), 5.22 (br d, J=16.9 Hz, 1H, H-15), 5.31 (ddddd, J=1.6, 1.6, 6.7, 6.7, 10.7 Hz, 1H, H-6), 5.50 (m, 1H, H-7), 5.51 (m, 1H, H-9), 5.52 (m, 1H, H-10), 5.56 (ddd, J=1.9, 2.3, 16.0 Hz, 1H, H-3), 5.67 (dddd, J=0.6, 6.6, 6.6, 15.1 Hz, 1H, H-12), 6.20 (br dd, J=10.4, 15.1 Hz, 1H, H-13), 6.25 (ddd, J=6.4, 6.4, 16.0 Hz, 1H, H-4), 6.44 (ddd, J=10.3, 10.4, 16.9, 1H, H-14); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 25.5 (t, C-8), 30.3 (t, C-11), 30.6 (t, C-5), 76.2 (d, C-1), 109.0 (d, C-3), 115.3 (t, C-15), 125.4 (d, C-6), 127.2 (d, C-9), 128.6 (d, C-7), 130.1 (d, C-10), 131.3 (d, C-13), 132.7 (d, C-12), 137.0 (d, C-14), 144.2 (d, C-4) ppm (a resonance for C-2 was not observed); eims *m/z* (rel. int.) $[M+H]^+$ 199 (0.1), $[M]^+$ 198 (0.1), 197 (0.5), 183 (2), 169 (3), 143 (15), 129 (45), 117 (30), 105 (40), 91 (100), 79 (80); hrms 199.1488 (calcd for $C_{15}H_{19}$, 199.1486).

Compound **2**.—(3*E*,6*Z*,9*Z*,12*E*)-Pentadeca-3,6,9,12-tetraen-1-yne [**2**]: an oil (5.9 mg, 0.008%); ir ν max (film) 3300, 3200, 2100, 1970, 965 cm^{-1}; uv λ max (EtOH) 221 nm (ϵ 16,400); ^1H nmr (C_6D_6, 300 MHz) δ 1.07 (t, J=7.5 Hz, 3H, H-15), 2.10 (br dq, J=7.5, 7.5 Hz, 2H, H-14), 2.67 (br dd, J=6.4, 6.8 Hz, 2H, H-5), 2.70 (dd, J=0.6, 2.2 Hz, 1H, H-1), 2.77 (br dd, J=7.2, 7.3 Hz, 2H, H-8), 2.85 (br dd, J=6.8, 6.8 Hz, 2H, H-11), 5.32 (ddddd, J=1.6, 1.6, 8.8, 9.0, 10.6 Hz, 1H, H-6), 5.48 (m, 1H, H-9), 5.53 (m, 1H, H-7), 5.53 (m, 1H, H-10), 5.54 (m, 1H, H-12), 5.57 (dd, J=2.6, 16.0 Hz, 1H, H-3), 5.62 (m, 1H, H-13), 6.26 (dddd, J=0.6, 6.4, 6.4, 16.0 Hz, 1H, H-4); ^{13}C nmr (C_6D_6, 75.5 MHz) 14.0 (q, C-15), 25.7 (t, C-8), 25.9 (t, C-14), 30.7 (t, C-5), 30.7 (t, C-11), 76.9 (d, C-1), 82.6 (s, C-2), 109.6 (d, C-3), 125.6 (d, C-6), 127.4 (d, C-9), 127.9 (d, C-12), 128.6 (d, C-7), 130.3 (d, C-10), 132.7 (d, C-13), 144.0 (d, C-4) ppm; eims *m/z* (rel. int.) $[M+H]^+$ 201 (1), $[M]^+$ 200 (0.1), 185 (0.5), 171 (3), 157 (2), 146 (3), 143 (6), 129 (15), 117 (15), 91 (35), 79 (35); hrms 200.1560 (calcd for $C_{15}H_{20}$, 200.1566).

Compound **8**.—α-Isobromocuparene [**8**]: an oil (4.4 mg, 0.006); $[\alpha]^{22}$D +37.7° ($CHCl_3$, c=0.22) [lit. (8) +91.0°]; ^1H nmr ($CDCl_3$, 300 MHz) δ 0.65 (s, 3H, H-7), 1.11 (s, 3H, H-8), 1.28 (d, J=1.0 Hz, 3H, H-6), 1.60 (ddd, J=3.8, 9.4, 13.1 Hz, 1H, H-5), 2.19 (dddd, J=3.8, 9.4, 12.8, 14.3 Hz, 1H, H-4), 2.33 (s, 3H, H-9), 2.53 (dddd, J=6.5, 9.3, 9.4, 14.3 Hz, 1H, H-4), 2.71 (br ddd, J=6.5, 12.8, 13.1 Hz, 1H, H-5), 4.46 (dd, J=9.4, 9.4 Hz, 1H, H-3), 7.12 (d, J=8.5 Hz, 1H, H-3′), 7.12 (d, J=8.5 Hz, 1H, H-5′), 7.27 (d, J=8.5 Hz, 1H, H-2′), 7.27 (d, J=8.5 Hz, 1H, H-6′); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 20.6 (q, C-8), 20.8 (q, C-7′), 22.2 (q, C-7), 25.4 (q, C-6), 31.3 (t, C-4), 33.6 (t, C-5), 47.8 (s, C-1), 48.5 (s, C-2), 63.6 (d, C-3), 126.5 (d, C-2′), 126.5 (d, C-6′), 128.6 (d, C-3′), 128.6 (d, C-5′), 135.5 (s, C-4′), 143.1 (s, C-1′) ppm; uv, ir, and ms data as previously reported (9).

Compound **7**.—(1*R**,3*S**)-3-Bromo-1,2,2-trimethyl-1-(4′-methylcyclohexa-1′,4′-dien)cyclopentane [**7**] was isolated as an oil (3.0 mg, 0.0045%); $[\alpha]^{22}$D +36.0° ($CHCl_3$, c=0.150; ir ν max (film) 2930, 1460, 1375 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 0.88 (s, 3H, H-7), 1.04 (s, 3H, H-6), 107 (s, 3H, H-8), 1.40 (ddd, J=5.8, 9.4, 12.7 Hz, 1H, H-5), 1.67 (br s, 3H, H-9), 2.04 (m, 1H, H-4), 2.34 (m, 1H, H-5), 2.38 (m, 1H, H-4), 2.64 (m, 2H, H-3′), 2.70 (m, 2H, H-6′), 4.30 (dd, J=8.9, 9.2 Hz, 1H, H-3), 5.41 (br s, 1H, H-5′), 5.55 (br s, 1H, H-2′); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 21.4 (q, C-7), 21.5 (q, C-8), 22.7 (q, C-7′), 23.7 (q, C-6), 28.3 (t, C-6′), 31.1 (t, C-4), 31.9 (t, C-3′), 33.7 (t, C-5), 47.1 (s, C-1*), 49.0 (s, C-2*), 64.4 (d, C-3), 118.9 (d, C-5′), 119.3 (d, C-2′), 130.6 (s, C-4′), 139.6 (s, C-1′) ppm (assignments with an asterisk are interchngeable); eims *m/z* (rel. int.) $[M]^+$ 284 (1), $[M]^+$ 282 (1), 203 (35), 147 (15), 133 (16), 119 (21), 105 (17), 69 (100); hrms 284.0954 (calcd for $C_{15}H_{20}{}^{81}Br$, 284.0964).

CONVERSION OF **7** TO **8**.—Compound **7** (3.0 mg) was dissolved in toluene and refluxed with 0.1 mg of palladium on activated carbon (10%). After 2 h the mixture was filtered and the solvent removed in vacuo to yield 2.8 mg of **8**.

Hplc separation of the combined vlc fractions 9 and 10, employing normal phase silica and hexane–Me_2CO–*t*-butylmethyl ether (15:3:2) as eluent afforded two C_{15} acetogenins, **6** and **3**.

Compound **6**.—(3*E*,6*R**,7*R**,9*S**,10*S**,12*R**,13*R**)-6,10:9,13-Bisepoxy-7-chloro-12-hydroxypentadeca-3-en-1-yne] [**6**]: an oil (1.7 mg, 0.0025%); $[\alpha]^{22}$D −67.8° ($CHCl_3$, c=0.09); uv λ max 223 (ϵ 11,800); ir ν max (film) 3440, 3290, 2915, 2100, 1450, 1065 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 0.98 (dd, J=7.5, 7.5 Hz, 3H, H-15), 1.70 (ddd, J=6.9, 7.5, 14.0 Hz, 2H, H-14), 1.78 (dd, J=3.3, 14.0 Hz, 1H, H-11), 2.05 (ddd, J=7.8, 9.1, 13.8 Hz, 1H, H-8), 2.18 (ddd, J=4.2, 6.8, 13.8 Hz, 1H, H-8), 2.25 (ddd, J=5.5, 9.8, 14.0 Hz, 1H, H-11), 2.46 (m, 2H, H-5), 2.83 (dd, J=0.7, 2.2 Hz, 1H, H-1), 2.95 (br d, J=10.6 Hz, 1H, 12-OH), 3.54 (ddd, J=2.5, 6.9, 6.9 Hz, 1H, H-13), 3.96 (ddd, J=4.2, 5.4, 7.8 Hz, 1H, H-7), 4.05

(br m, 1H, H-12), 4.08 (ddd, J=5.4, 5.6, 6.5 Hz, 1H, H-6), 4.11 (ddd, J=2.6, 3.3, 9.8 Hz, 1H, H-10), 4.41 (ddd, J=2.6, 6.8, 9.1 Hz, 1H, H-9), 5.59 (dddd, J=1.5, 1.6, 2.2, 15.9 Hz, 1H, H-3), 6.22 (dddd, J=0.7, 7.4, 7.4, 15.9 Hz, 1H, H-4); ^{13}C nmr ($CDCl_3$, 75.5 MHz) 10.5 (q, C-15), 21.8 (t, C-14), 35.1 (t, C-11), 36.6 (t, C-5), 38.1 (t, C-8), 58.2 (d, C-7), 71.0 (d, C-12), 77.2 (d, C-1), 77.9 (d, C-10), 79.2 (d, C-9), 81.7 (s, C-2), 85.7 (d, C-13), 86.0 (d, C-6), 112.4 (d, C-3), 139.9 (d, C-4) ppm; eims *m/z* (rel. int.) $[M]^+$ 286 (0.1), $[M]^+$ 284 (0.1); 249 (1), 221 (15), 219 (48), 133 (30), 115 (67), 71 (100); hrms 249.1358 (calcd for $C_{15}H_{21}O_{37}$, 249.1491).

Compound **3**.—(3*E*,6*R**,7*R**,9*R**,10*S**)-6,9:7,10-Bisepoxy-13-chloro-12-hydroxypentadeca-3,14-dien-1-yne[**[3]**: an oil (5.7 mg, 0.0085%); $[\alpha]^{22}$ D+53.4° ($CHCl_3$, c=0.29); ir ν max (film) 2900, 1190, 1150, 1065, 655 cm^{-1}; uv λ max (EtOH) 223 nm (ϵ 8180); ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.48 (m, 2H, H-11), 1.89 (br s, 2H, H-8), 2.44 (m, 2H, H-5), 2.80 (dd, J=0.5, 2.2 Hz, 1H, H-1), 3.84 (ddd, J=1.1, 7.1, 7.1 Hz, 1H, H-6), 3.95 (m, 1H, H-12), 4.07 (dd, J=5.2, 8.6 Hz, 1H, H-10), 4.34 (br s, 1H, H-7), 4.34 (br s, 1H, H-9), 4.38 (m, 1H, H-13), 5.29 (d, J=10.1 Hz, 1H, H-15), 5.36 (d, J=16.9 Hz, 1H, H-15), 5.60 (dd, J=2.2, 11.1 Hz, 1H, H-3), 5.93 (ddd, J=8.6, 10.1, 16.9 Hz, 1H, H-14), 6.22 (dddd, J=0.5, 7.3, 7.3, 16.1 Hz, 1H, H-4); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 284 (0.1), $[M]^+$ 282 (0.1), 266 (0.1), 264 (0.3), 247 (1), 229 (2), 219 (7), 217 (18), 181 (11), 163 (7), 143 (15), 121 (30), 69 (90); hrms 217.0523 (calcd for $C_{10}H_{14}{}^{35}ClO_3$, 217.0632).

Hplc separation of the combined vlc fractions 11 and 12 employing normal phase silca and hexane–Me_2CO–*t*-butylmethyl ether (15:3:2) as eluent afforded a single pure compound, **5**.

Compound **5**.—(3*E*,6*R**,7*R**,9*R**,10*S**,13*E*)-6,9:7,10-Bisepoxy-15-chloro-12-hydroxypentadeca-3,13-dien-1-yne] **[5]**: an oil (6.1 mg, 0.009%); $[\alpha]^{22}$ D+79.9° ($CHCl_3$, c=0.31); uv λ max 215 nm (ϵ 18,220); ir ν max (film) 3410, 3290, 2920, 2100, 1730, 1245, 1075 cm^{-1}; ^{1}H nmr (C_6D_6, 300 MHz) δ 1.13 (ddd, J=5.0, 8.3, 14.1 Hz, 1H, H-11), 1.20 (ddd, J=3.5, 7.6, 14.1 Hz, 1H, H-11), 1.40 (dd, J=2.5, 10.2 Hz, 1H, H-8), 1.46 (dd, J=2.8, 10.2 Hz, 1H, H-8), 2.30 (dddd, J=1.4, 6.7, 7.5, 13.9 Hz, 1H, H-5), 2.46 (dddd, J=1.7, 6.9, 7.0, 13.9 Hz, 1H, H-5), 2.66 (d, J=2.3 Hz, 1H, H-1), 3.48 (ddd, J=1.2, 6.7, 6.9 Hz, 1H, H-6), 3.69 (ddd, J=1.1, 1.1, 6.9 Hz, 1H, H-15), 3.83 (d, J=2.8 Hz, 1H, H-7), 4.03 (dd, J=5.0, 7.6 Hz, 1H, H-10), 4,04 (d, J=2.5 Hz, 1H, H-9); 4.07 (br m, 1H, H-12), 5.45 (dddd, J=1.1, 1.1, 5.3, 15.5 Hz, 1H, H-13), 5.63 (dddd, J=1.4, 1.7, 2.3, 16.0 Hz, 1H, H-13), 5.66 (dddd, J=1.1, 1.1, 6.9, 15.5 Hz, 1H, H-14), 6.29 (ddd, J=7.0, 7.5, 16.0 Hz, 1H, H-4); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M\text{-}H_2O]^+$ 266 (1), $[M\text{-}H_2O]^+$ 264 (2), 229 (2), 217 (7), 173 (5), 171 (16), 162 (17), 133 (20), 115 (77), 105 (75), 91 (38), 69 (71); hrms 264.0900 (calcd for $C_{15}H_{17}O_2{}^{35}Cl$, 264.0917).

Hplc separation of vlc fraction 13 using normal phase silica and hexane containing 25% Me_2CO as eluent afforded **4** as a pure compound.

Compound **4**.—(3*E*,6*R**,7*R**,9*R**,10*S**)-6,9:7,10-Bisepoxy-12,13-dihydroxypentadeca-3,14-dien-1-yne [**4**]: an oil (2.0 mg, 0.003%); $[\alpha]^{22}$ D+47.0° ($CHCl_3$, c=0.1); uv λ max 223 nm (ϵ 9400); ir ν max (film) 3400, 3280, 2920, 2100, 1720, 1070 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 1.44 (m, 2H, H-11), 1.87 (m, 1H, H-8), 1.91 (m, 1H, H-8), 2.05 (br s, 1H, 13-OH), 2.27 (br s, 1H, 12-OH), 2.44 (m, 2H, H-5), 2.80 (dd, J=0.5, 2.3, 1H, H-1), 3.82 (m, 1H, H-12), 3.84 (ddd, J=1.1, 7.1, 7.1 Hz, 1H, H-6), 4.07 (dd, J=5.2, 8.4 Hz, 1H, H-10), 4.15 (m, 1H, H-13), 4.34 (br s, 1H, H-9), 4.35 (br s, 1H, H-7), 5.27 (ddd, J=1.2, 1.5, 10.5 Hz, 1H, H-15), 5.34 (ddd, J=1.2, 1.4, 16.9 Hz, 1H, H-15), 5.59 (ddd, J=1.4, 1.4, 2.3, 16.0 Hz, 1H, H-3), 5.89 (ddd, J=6.4, 10.5, 16.9 Hz, 1H, H-14), 6.22 (dddd, J=0.6, 7.6, 7.6, 16.0 Hz, 1H, H-4); ^{13}C nmr see Table 1; eims, *m/z* (rel. int.) $[M]^+$ 264 (0.1), 199 (10), 181 (15), 121 (25), 105 (27), 91 (32), 69 (100); hrms 199.1002 (calcd for $C_{10}H_{15}O_4$, 199.0970).

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