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Two New Chamigranes From an Hawaiian Red Alga, *Laurencia cartilaginea*

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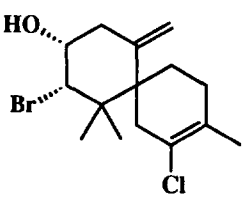
Abstract: From the red alga *Laurencia cartilaginea*, six halogenated sesquiterpenes were isolated. Two new chamigranes, ma'ilione (**5**) and *allo*-isoobtusol (**6**) were identified by spectroscopic methods. Their cytotoxicity profiles are reported. Copyright © 1996 Elsevier Science Ltd

The red algal genus *Laurencia* has long been known as a reliable and prolific producer of secondary metabolites, paricularly of C_{15} acetogenins and of sesquiterpenes.[2] We had an opportunity to collect on the Wai'anae coast of O'ahu *L. cartilaginea*,[3] which had not been examined previously. The lipid extracts of *L. cartilaginea* have yielded four halogenated sesquiterpenes: elatol (**1**) previously known from *L. elata*[4] and *L. obtusa*,[5,6] [1(15)*Z*,2*Z*,4*R*,8*S*,9*R*]-8,15-dibromochamigra-1(15),2,11(12)-trien-9-ol(**2**) from *L. majuscula*[7,8,9] and from a sea hare *Aplysia dactylomela*;[10] [1(15)*E*, 2*Z*, 4*R*, 8*S*, 9*R*]-8, 15-dibromochamigra-1(15),2,11(12)-trien-9-ol (**3**) from *L. majuscula*;[11] isoobtusadiene (**4**) from *L. majuscula*[11] and *L. obtusa*,[12] and two new chamigrane sesquiterpenes. Freshly collected algae (1.3 kg, wet) were freed from debris and extracted with 3L



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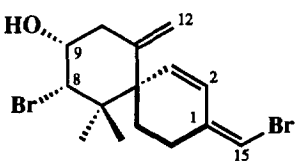

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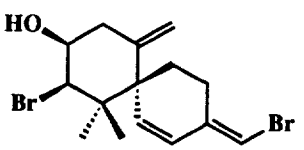

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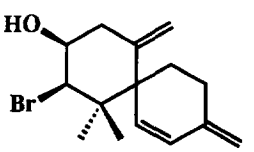

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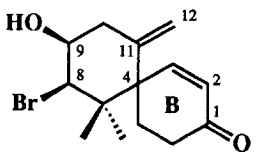
of MeOH, which yielded 11 g of residue. A 1.0 g portion of the crude residue was separated by high-speed countercurrent chromatography (hex/MeCN/CH_2Cl_2, 10:7:3 lower mobile phase) into four fractions. The fractions displayed moderate activity (IC_{50} 1-5 μg/mL) in the P-388, A-549, HT-29, and MEL-28 assays, which were used to guide the isolation. The third fraction (131.9 mg) was subjected to repeated HPLC on silica (hex/EtOAc, 8:2) to yield **1**. The technique was repeated with hex/EtOAc (85:15) on fractions 2 and 4 to yield metabolites **2**, **3**, **4**, and two new halogenated sesquiterpenes, 8-bromo-9-hydroxychamigra-2(3),11(12)-dien-1-one (**5**) and 2-chloro-1,8-dibromochamigr-11(12)-en-9-ol (**6**). We propose the trivial name ma'ilione[13] for compound **5**. Compound **6** is a diastereomer of isoobtusol;[14] hence **6** is *allo*-isoobtusol.



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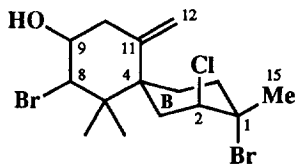

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Ma'ilione is a norsesquiterpene with a molecular formula of $C_{14}H_{19}BrO_2$, as determined by HREIMS. The missing carbon is replaced by a carbonyl resonating at δ199.0; this assignment is supported by a UV maximum at 241 nm and IR bands at 1695 and 1650 cm^{-1},which are attributable to a conjugated carbonyl.[15] ^{13}C NMR data (Table 1) of **5** revealed the presence of three additional quarternary carbons resonating at δ51.9 (C4), δ42.3 (C7), and at δ141.7 (C11). Furthermore, the peaks at δ51.9 and δ42.3 are characteristics of spiro and quarternary carbon bearing geminal methyl groups in chamigranes.[7] The ^1H NMR spectrum of **5** showed absorptions for two quarternary methyl groups, δ1.06 (s) and δ1.31 (s), and four olefinic protons, δ7.04 (1H, dd, *J*=2.1, 1.9 Hz), δ6.12 (1H, d, *J*=11.2 Hz), δ4.86 (s). and δ5.19 (s). Ring B of **5** is similar to that of majusculone,[9] a spiro diketone isolated from *L. majuscula*.

Compound **6** (3.2 mg) has a molecular formula of $C_{15}H_{23}Br_2ClO$ based on HREIMS. Interpretation and detailed comparison of its NMR data (Table 1) with reported values for a known metabolite, isoobtusol (**7**)[16,17] revealed many similarities. Significant chemical shift differences were observed for C1, C2, and C15: δ73.2, δ57.1, and δ33.1 in **6** compared to δ71.0, δ65.2, and δ25.7 in isoobtusol (**7**). We made a complete analysis based on HMBC, HMQC, and COSY experiments to map the correct locations of the C-H functionalities on ring B, which left Cl and Br to be assigned. The *L. cartilaginea* metabolite **6** was also investigated by nOe (Fig. 1); irradiation of Me at δ1.75 enhanced the proton signal at δ4.43, which indicates that Br and Cl are diaxial.

Table 1. ^{13}C and ^{1}H NMR Data for Compounds **5** and **6** ($CDCl_3$, 500 MHz)

	5		**6**	
#C	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**
1	199.0 (s)	–	73.2 (s)	–
2	131.1 (d)	7.04 (1H, dd, *J*=10.9, 2.0 Hz)	57.1 (d)	4.43 (1H, br dd, *J*=4.0 Hz)
3	158.2 (d)	6.12 (1H, d, *J*=11.2 Hz)	33.8 (t)	2.78 (1H, dd, *J*=4.0, 16.0 Hz) 3.09 (1H, br d, *J*=16.0 Hz)
4	51.9 (s)	–	44.0 (s)	–
5	26.9 (t)	2.19 (2H, m)	24.3 (t)	1.74 (1H, dq, *J*=14.0, 3.5 Hz) 2.04 (1H, dt, *J*=3.5, 13.7 Hz)
6	34.1 (t)	2.36 (2H, m)	32.4 (t)	1.79 (1H, br dq, *J*=13.3, Hz) 2.39 (1H, td, *J*=3.4, 13.3 Hz)
7	42.3 (s)	–	43.7 (s)	–
8	68.8 (d)	4.59 (1H, d, *J*=2.9 Hz)	76.3 (d)	4.41 (1H, dd, *J*=1.4, 3.4 Hz)
9	71.7 (d)	4.20 (1H, m)	69.6 (d)	3.68 (1H, br dt, *J*=11.7, 3.7 Hz)
10	38.4 (t)	2.73 (1H, m) 2.76 (1H, m)	39.4 (t)	2.45 (1H, dd, *J*=3.7, 12.8 Hz) 2.72 (1H, t, *J*=12.3 Hz)
11	141.7 (s)	–	147.2 (s)	–
12	117.8 (t)	4.86 (1H, s) 5.19 (1H, s)	114.7 (t)	4.97 (1H, s) 5.20 (1H, s)
13	26.3 (q)	1.06 (3H,s)	25.3 (q)	1.04 (3H, s)s
14	21.4 (q)	1.31 (3H, s)	24.8 (q)	1.31 (3H, s)
15			33.1 (q)	1.75 (3H, s)

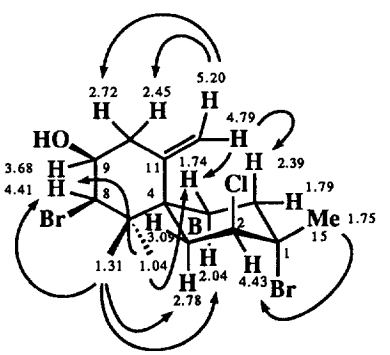
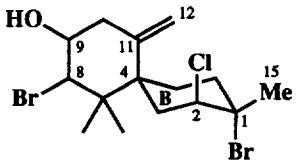


Fig. 1. Perspective representation of **6** with key nOe interactions.

Metabolite **8** ([1*S*, 2*S*, 4*R*, 8*R*, 9*S*)-1-chloro-2, 8-dibromochamigr-11(12)-en-9-ol)[11] exhibits comparable NMR spectral signals (Table 2) as **6** and **7** but has reversed halogen positions. These differences in chemical shifts at C1 and C2 can be explained by interchanging halogen substituents. Compound **6** and **7** have identical NMR spectral properties and comparable IR values but are antipodal in optical rotation, $[\alpha]_D$ -33.8° for **6** and

$[\alpha]_D$ +33.0° for **7**. Metabolite **6** is *allo*-isoobtusol.[18]



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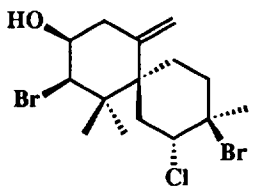

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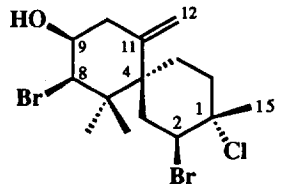

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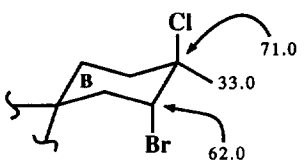
Table 2. ^{13}C NMR Chemical Shifts for Compounds **6**, **7**, and **8**

C#	6	7	8
1	73.2	71.0	71.6
2	57.1	65.2	60.9
3	33.8	34.0	33.8
4	44.0	44.0	50.9
5	24.3	33.1	25.5
6	32.4	33.4	38.6
7	43.7	43.7	44.1
8	76.3	76.2	70.4
9	69.6	69.7	71.8
10	39.4	39.2	38.6
11	147.2	147.5	141.5
12	114.7	113.8	117.6
13	25.3	25.3	24.1
14	24.8	24.3	20.8
15	33.1	25.7	24.1

Halogen placement in such systems has been studied by x-ray crystallography,[14,17] by ^{13}C NMR analysis,[17,19,20] and by comparison between calculated (**9**) and observed (**10**) chemical shifts of known



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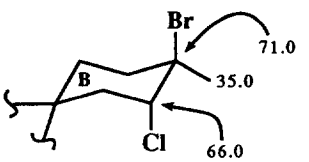

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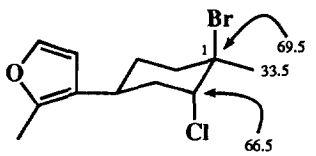
chamigrane-type metabolites with identical ring B systems.[17,20] The chemical shift at δ33.1 in **6** therefore is assigned to $Me_{eq}15$,while the signal at δ25.7 in **7** is assignable to $Me_{ax}15$. The relationship between the carbon chemical shifts of known metabolites isofurocaespitane (**11**)[17] and isocaespitol (**12**)[21] have definitely established that the crucial methyl functionality at C1 is in equatorial conformation. Position of the halogens is determined by their secondary nature (**9** and **10**). The corresponding chemical shifts of **6** parallel those of the model compounds.



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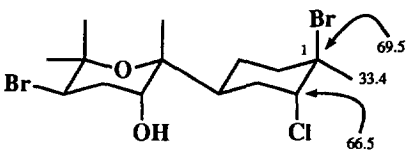

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Natural products **1-6** were screened for cytotoxicity (Table 3). All metabolites have shown remarkable results against cancer cell lines at low concentrations, especially to HT-29 (Human Colon Carcinoma). Known compounds **2** and **4** were also screened by NCI;[22] they displayed cytotoxicity to colon, lung, prostrate, and melanoma cell lines respectively.

Table 3. Cytotoxicity Activity Results

Cell Lines	1	2	3	4	5	6
P-388 (IC_{50} μg/mL)	1.0	1.0	1.0	1.0	5.0	5.0
A-549 (IC_{50} μg/mL)	0.1	1.0	1.0	1.0	5.0	1.0
HT-29 (IC_{50} μg/mL)	0.1	0.025	0.025	0.25	0.5	0.25
MEL-28 (IC_{50} μg/mL)	0.1	1.0	1.0	1.0	10.0	1.0

EXPERIMENTAL PART

NMR spectra were determined on a General Electric GN Omega instrument operating at 500 and 300 MHz for ^1H and 75 for ^{13}C, respectively. IR spectra were measured on a Perkin-Elmer/1420 Ratio Recording

Infrared spectrophotometer. UV spectra were determined in MeOH on a Hewlett Packard 8452A spectrophotometer. Optical rotation was determined on a JASCO DIP-370 digital polarimeter. Mass spectra were obtained with a VG 70/SE mass spectrometer. Analytical separations were performed on precoated plates with Si gel $60F_{254}$ (Merck, Darmstadt). Counter-current chromatography was carried out using the Ito Multi-Layer Coil Separator-Extractor (PC Inc., Potomac, MD). Normal-phase HPLC was carried out with MICROSORB (Si 80-199-C5) column. Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

The red alga was collected at a depth of 0.5 m at Ma'ili Pt. Park off the Wai'anae coast of O'ahu on July 8, 1994. It was identified by Prof. I. A. Abbott as *Laurencia cartilaginea*. The wet specimen (1.3 kg) was extracted with MeOH (3L); a residue of 11.0 g was obtained after solvent removal. A portion (1.0 g) of the crude residue was separated with bioassay-guided fractionation (P-388, A-549, HT-29, and MEL-28 assays) by high speed counter-current chromatography with solvent system of hex/MeCN/CH2Cl2, 10:7:3, lower mobile phase. Active fractions 2,3, and 4 were subjected to repeated HPLC on Si, with a solvent system of hex/EtOAc (8:2), then hex/EtOAc (85:15) which furnished **1** (111.8 mg) , **2** (56.0 mg) and **3** (27.3 mg) , and compounds **4** (2.0 mg), **5** (0.4 mg) and **6** (3.2 mg) as oils.

Elatol (**1**, 111.8 mg) was obtained as the major component of *L. cartilaginea* by repeated HPLC on Si as a colorless oil. Its ^{1}H, ^{13}C NMR, mass, and IR spectra were identical with those in the literature.[4,6]

[1(15)*Z*, 2*Z*, 4*R*, 8*S*, 9*R*]-8, 15-dibromochamigra-1(15), 2, 11(12)-trien-9-ol (**2**, 56.0 mg) was isolated as a clear oil by repeated HPLC. It was identified by detailed comparison of its ^{1}H, ^{13}C NMR, mass, and IR spectral data with literature values.[7]

[1(15)*E*, 2*Z*, 4*R*, 8*S*, 9*R*]-8, 15-dibromochamigra-1(15), 2, 11(12)-trien-9-ol (**3**, 27.3 mg) was obtained by repeated HPLC on Si as a clear oil. Its ^{1}H, ^{13}C NMR, mass, and IR spectra were identical with those in the literature.[11]

Isoobtusadiene (**4**, 2.0 mg) was isolated by repeated HPLC on Si as a colorless oil. Its ^{1}H, ^{13}C NMR, mass, and IR spectral data were consistent with those reported in the literature.[11,12]

Ma'ilione [8-bromo-9-hydroxychamigra-2(3), 11(12)-dien-1-one, (**5**, 0.4 mg)] pale yellow oil; $[\alpha]_D$ -100° ($CHCl_3$, *c*=0.20); UV(MeOH) λ_{max} 228 nm (ε 8769); IR($CHCl_3$) ν_{max} 3000, 1695, 1650 cm^{-1}; ^{13}C NMR (500 MHz, $CDCl_3$); see Table 1; ^{1}H NMR (500 MHz, $CDCl_3$); see Table 1; HREIMS observed *m/z* 298.0590, required 298.0569 (Δ 2.1 mmu). All assignments were confirmed by COSY, HMQC, and HMBC experiments.

***allo*-Isoobtusol** [(-)2-chloro-1, 8-dibromochamigr-11(12)-en-9-ol, (**6**, 3.2 mg)] pale yellow oil; $[\alpha]_D$ -33.8°

($CHCl_3$, c=1.50); IR($CHCl_3$) ν_{max} 3508, 3000, 1650 cm^{-1}; ^{13}C NMR (500 MHz, $CDCl_3$); see Table 1; ^1H NMR (500 MHz, $CDCl_3$); see Table 1; HREIMS observed *m/z* 411.9781, required 411.9804 (Δ-2.3 mmu). All assignments were confirmed by COSY, HMQC, HMBC and nOe experiments.

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