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Cytotoxic cuparene sesquiterpenes from *Laurencia microcladia*

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Abstract—Three new cuparene sesquiterpenes **1**–**3** were isolated from the organic extract of the red alga *Laurencia microcladia*, collected at Chios island in the North Aegean Sea, Greece. The structures and the relative stereochemistry of the compounds are proposed on the basis of their spectral data. Metabolite **2** shows an unprecedented (for the cuparene class of sesquiterpenes) migration of the C-1 methyl group. All metabolites **1**–**3** were found to exhibit significant cytotoxic activity against two lung cancer cell lines.

The red algae belonging to the genus *Laurencia* (Ceramiales, Rhodomelaceae) have been proven to be rich sources of secondary metabolites. The majority of these metabolites are characterized by their relatively high degree of halogenation.[1–3] Their ecological roles have not been clearly elucidated, but it is suggested that they function as chemical defenses against marine herbivores.[4,5] Despite the fact that *Laurencia* has been studied extensively with respect to secondary metabolite chemistry, new studies on members of this genus frequently lead to the isolation of novel intriguing structures, especially terpenoids and C_{15}-acetogenins.[6,7]

As part of our ongoing program aimed at the isolation of biologically active compounds from marine organisms of the Greek seas,[8–10] we investigated the secondary metabolite content of the red alga *Laurencia microcladia*, collected from the south coast of Chios island. We herein describe the isolation and structure elucidation of the new sesquiterpenes **1**–**3**.

The collected alga specimens were initially freeze-dried (291.4 g dry weight) and then exhaustively extracted with mixtures of CH_2Cl_2/MeOH (3:1) at room temperature. The extract was concentrated to give a dark green residue (12.2 g), which was subjected to vacuum column chromatography (VCC) on silica gel, using cyclohexane with increasing amounts (10%) of EtOAc as the mobile phase. Following a combination of chromatographic techniques, including VCC, gravity column and finally HPLC purifications, compounds **1** (4.3 mg), **2** (3.0 mg), and **3** (1.5 mg) were isolated in pure form.

Metabolite **1**, a bromo sesquiterpene ether, was isolated as a colorless oil, $[\alpha]_D^{20}$ −29.00 (*c* 0.1, CH_2Cl_2). The HRFAB-MS measurements supported the molecular formula $C_{15}H_{19}OBr$ (*m*/*z* 294.0611 [M^+]) and the M^+ peaks in the EI-MS spectrum, at *m*/*z* 294 and 296 with relative intensities 1/1, revealed the presence of one bromine atom in the molecule. The presence of a substituted benzene ring was evident from the UV spectrum,[11] which had maxima at 275 and 285 nm and the IR spectrum with absorbances at 1648 and 1508 cm^{-1}. The IR absorption at 1241 cm^{-1} and the absence of an absorption band for hydroxyl or carbonyl groups indicated that the oxygen atom was involved in an ether linkage. The ^{13}C NMR spectrum of **1** (Table 1) showed signals for 15 carbons with the multiplicities of the carbon signals determined from the DEPT spectrum as: six quaternary, three methine, two methylene, and four methyl carbon atoms. The ^1H and ^{13}C NMR spectra of **1** confirmed the presence of the aromatic ring (δ 6.56/117.6, 7.18/127.8). Since **1** has six degrees of unsaturation, with no other additional double bonds, it must contain two other rings, one of which is the ether-containing ring

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Table 1. ^{1}H and ^{13}C NMR data (400 and 50.3 MHz, respectively) for metabolites **1**–**3**

	Metabolite **1**				Metabolite **2**			Metabolite **3**		
	^{1}H (δ)	^{13}C (δ)	HMBC		^{1}H (δ)	^{13}C (δ)	HMBC	^{1}H (δ)	^{13}C (δ)	HMBC
C-1	—	45.9	—	C-1	—	147.1	—	—	54.3	—
C-2	—	41.2	—	C-2	—	49.3	—	—	137.1	—
H-3	4.10 (d, 5.1)	86.3	C-2, C-7	H-3	2.08 (m)	44.8	—	—	131.8	—
H-4	2.09 (m)	30.0	—	H-4	2.53 (m) 2.06 (m)	38.3	C-2	1.93 (m) 2.29 (m)	35.7	—
H-5	1.88 (m)	41.3	C-2, C-4, C-6	H-5	5.75 (br s)	131.1	C-2, C-3, C-4	1.38 (m) 1.72 (m)	41.2	—
C-6	—	133.1	—	C-6	—	123.1	—	—	148.6	—
C-7	—	151.8	—	C-7	—	152.1	—	6.62 (s)	112.2	C-11
H-8	6.56 (s)	117.6	C-6, C-7, C-10, C-15	H-8	6.82 (s)	117.0	C-6, C-10, C-15	—	153.4	—
C-9	—	136.3	—	C-9	—	137.9	—	—	130.4	—
C-10	—	114.5	—	C-10	—	114.0	—	7.00 (d, 7.8)	129.0	C-6, C-8, C-15
H-11	7.18 (s)	127.8	C-1, C-7, C-9, C-10	H-11	7.17 (s)	131.9	C-1, C-7, C-9	6.71 (d, 7.8)	118.7	C-7
H-12	0.89 (s)	18.1	C-1, C-2, C-3, C-13	H-12	0.87 (s)	20.5	C-1, C-2, C-3, C-13	1.35 (s)	10.1	C-2, C-3
H-13	0.97 (s)	20.4	C-1, C-2, C-3, C-12	H-13	0.97 (s)	26.1	C-1, C-2, C-3, C-12	1.68 (s)	14.3	C-2, C-3, C-4
H-14	1.22 (s)	14.9	C-1, C-5, C-6	H-14	1.01 (d, 6.8)	14.2	C-3, C-4	1.35 (s)	26.7	C-1, C-5, C-6
H-15	2.26 (s)	22.4	C-8, C-9, C-10	H-15	2.32 (s)	22.7	C-8, C-9, C-10	2.19 (s)	14.9	C-9, C-8
				OH-C-7	5.33 (s)		C-7, C-8	4.63 (s)		

Chemical shifts are expressed in ppm. J values in parentheses are in Hz.

and the other a five-membered carbocyclic ring. Signals corresponding to four tertiary methyl groups appeared at δ 0.89/18.1, 0.97/20.4, 1.22/14.9 and 2.26/22.4. A doublet at δ 4.10 was attributed to a proton on carbon C-3 (δ 86.3), participating in the ether bridge. The ether bridge was placed on carbons C-7 and C-3 because of the heteronuclear correlation between H-3 (δ 4.10) and C-7 (δ 151.8). The correlation of signals at δ 0.89 (H-12)/0.97 (H-13) with those at 45.9 (C-1), 41.2 (C-2), and 86.3 (C-3) confirmed the position of the *gem*-dimethyl groups on C-2. The relative stereochemistry of **1** was assigned on the basis of NOESY experiments. The strong NOE correlations between H-14/H-13 and between H-13/H-3 determined the stereochemistry at C-1 and C-3. The ^{1}H and ^{13}C NMR data of compound **1** are in good agreement with reported values for the debromo-analogue **4** isolated previously from *Laurencia okamurai*.[12] In view of the above-mentioned data and considering the biosynthetic relations, the proposed structure for ether metabolite **1** is shown in Figure 1.

Metabolite **2**, a bromo sesquiterpene phenol, obtained as a colorless oil with $[\alpha]_D^{20}$ +5.00 (*c* 0.06, CH_2Cl_2), showed LREI-MS signals at *m/z* 294/296 [M^+], with relative intensities 1/1, suggesting the presence of one bromine atom. The HRFAB-MS measurements supported the molecular formula $C_{15}H_{19}OBr$ (*m/z* 294.0628 [M^+]). The presence of a benzene ring was evident from the IR spectrum, which showed absorbances at 1642 and 1465 cm^{-1}. The intense sharp absorptions at v_{max} 3428 and 1157 cm^{-1} indicated the presence of a hydroxyl functionality in the molecule.[13] The ^{13}C NMR spectrum of **2** (Table 1) showed signals for 15 carbons. Multiplicities for the carbon signals were determined from the DEPT spectra as: six quaternary, four methine, one methylene and four methyl carbons. The ^{1}H and ^{13}C NMR spectra displayed resonances for a secondary methyl (δ 1.01 d/14.2), one aromatic methyl (δ 2.32/22.7), two quaternary methyls (δ 0.87/20.5 and δ 0.97/26.1), an olefinic proton (δ 5.75/131.1), two aromatic protons (δ 6.82/117.0 and δ 7.17/131.9), one methine (δ 2.08/44.8), one methylene (δ 2.53, 2.06/38.3), and one exchangeable proton (δ 5.33). The position of the olefinic proton of the trisubstituted double bond at C-5 was determined from correlations between H-5/C-2, H-5/C-3, and H-5/C-4. Moreover, the correlation of the vinyl carbon (δ 147.1) with the aromatic proton H-11 (δ 7.17) as observed in the HMBC spectrum confirmed the position of the double bond between C-1 and C-5. The correlation of signals at δ 0.87 (H-12)/0.97 (H-13) with 147.1 (C-1), 49.3 (C-2), and 44.8 (C-3) determined the position of the *gem*-dimethyl groups on C-2, vicinal to the secondary methyl, located on C-3. Moreover, NOE correlations between the aromatic proton H-11 and the geminal methyls C-12, C-13 further supported the position of the methyls (C-12 and C-13) on C-2. Combination of these data led to the assignment of the structure as shown in Figure 1. To the best of our knowledge, this is the first report of a cuparene sesquiterpene with a double bond between C-1 and C-5 resulting from methyl migration on either the C-2 or C-3 position. Metabolite **2** could have potentially arisen from rearrangement of compound **1**, but the fact that

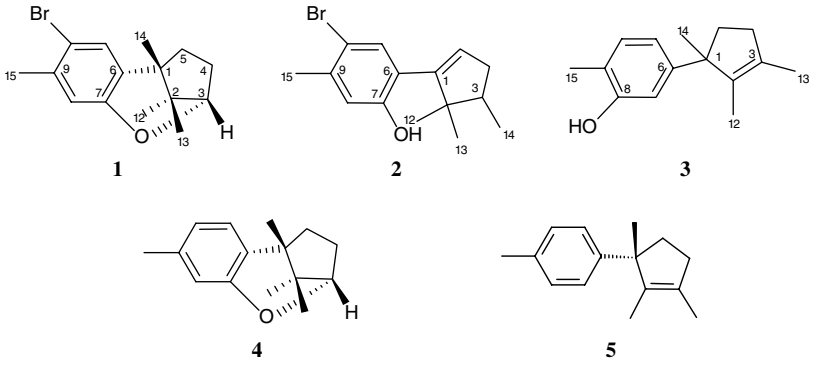

Figure 1. Structures of metabolites **1**–**5**.

no conversion or instability of metabolite **1** was observed during the chromatographic separations supports **2** as an algal secondary metabolite.

Metabolite **3** was isolated as a colorless oil, with $[\alpha]_D^{20}$ +40.00 (*c* 0.07, CH_2Cl_2). The HRFAB-MS measurements supported the molecular formula $C_{15}H_{19}O$ (*m/z* 215.1443 $[M-1]^+$). The presence of a substituted benzene ring was suggested by the UV spectrum,[14] which had maxima at 274 and 280 nm. The IR absorptions at ν_{max} 3422 and 1639 cm^{-1} were attributed to a hydroxyl and a double bond, respectively. The ^{13}C NMR spectrum displayed fifteen signals (Table 1) and their multiplicities were determined from the DEPT spectra as: six quaternary (two olefinic, three aromatic, and one aliphatic), three methines, two methylenes, and four methyl carbons. The ^1H NMR spectrum exhibited absorptions at δ 6.62 (s, 1H), 6.71 (d, 1H), and 7.00 (d, 1H) due to aromatic protons, an aromatic methyl group at 2.19 (s, 3H), a tertiary methyl group at δ 1.35, and two vinyl methyls at δ 1.35 and 1.68. The vinyl methyl at δ 1.35 appears unexpectedly at high field frequency probably due to the magnetic anisotropy exerted by the benzene ring.[15] Moreover, the lack of any NOE between H-7/H-11 and the methyl group at δ 1.68 supported its position on C-13. Comparison of the NMR data of **3** with reported values for isolaurene **5** was in accordance with **3** being its hydroxyl derivative.[16,17] The chemical shift of the aromatic methyl group at δ 2.19/14.9 at higher field compared to isolaurene (δ 2.30/20.9) supported the position of the hydroxyl functionality on C-8. Furthermore, an HMBC correlation between H-15 (δ 2.19) and C-8 (δ 153.4) further confirmed the position of the hydroxyl group. In view of the above-mentioned data, the proposed structure for metabolite **3** is shown in Figure 1.

The cytotoxicities of compounds **1**–**3** were assayed against NSCLC-N6 and A549 lung cancer cell lines. Metabolite **1** showed moderate cytotoxicity: IC_{50} = 196.9 and 242.8 μM against NSCLC-N6 and A549 cancer cell lines, respectively. Metabolites **2** and **3** showed stronger levels of activity with: IC_{50} = 73.4 μM (NSCLC-N6) and 52.4 μM (A549) and IC_{50} = 83.7 μM (NSCLC-N6) and 81.0 μM (A549), respectively. The higher cytotoxicity of metabolites **2** and **3** could be attributed to the phenolic hydroxyl and/or the presence of the double bond in the five-membered ring. The presence of bromine in these molecules does not seem to affect significantly their activity according to our results.

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11. Spectral data of compound **1**: $[\alpha]_D^{20}$ −29.00 (*c* 0.1, CH_2Cl_2); HRFAB-MS: *m/z* $[M]^+$; 294.0611 (294.0619 calculated for $C_{15}H_{19}O^{79}Br$); IR (KBr): ν_{max} 1648, 1541, 1508, 1391, 1376, 1241, 1162, 1041, 812 cm^{-1}; UV $\lambda_{max}^{CH_2Cl_2}$ (nm) ($\log\varepsilon$) 237 (3.02), 275 (2.42), 285 (2.56), 295 (2.44). NMR data are shown in Table 1.
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13. Spectral data of compound **2**: $[\alpha]_D^{20}$ +5.00 (*c* 0.06, CH_2Cl_2); HRFAB-MS: *m/z* $[M]^+$; 294.0628 (294.0619 calculated for $C_{15}H_{19}O^{79}Br$); IR (KBr): ν_{max} 3428, 2952, 2921, 2861, 1642, 1465, 1157 cm^{-1}; UV $\lambda_{max}^{CH_2Cl_2}$ nm ($\log\varepsilon$) 237 (3.19), 290 (2.92). NMR data are shown in Table 1.
14. Spectral data of compound **3**: $[\alpha]_D^{20}$ +40.00 (*c* 0.07, CH_2Cl_2); HRFAB-MS: *m/z* $[M-1]^+$; 215.1443 (215.1437 calculated for $C_{15}H_{19}O$); IR (KBr): ν_{max} 3422, 1639,

725 cm^{-1}; UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε) 235 (2.88), 274 (2.67), 280 (2.55). NMR data are shown in Table 1.

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