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New sesquiterpenes from the red alga *Laurencia microcladia*

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Abstract—Three new aromatic sesquiterpenes (**1**, **2**, and **4**), one new dimeric sesquiterpene of the cyclolaurane-type (**3**), one sesquiterpene alcohol of bisabolene type (**8**) along with three previously reported metabolites (**5**–**7**), were isolated from the organic extracts of *Laurencia microcladia*, collected from the Chios island in the North Aegean Sea. The structures of the new natural products, as well as their relative stereochemistries, were established by means of spectral data analyses, including 2D experiments. The cytotoxicity of the isolated metabolites was evaluated against five human tumor cell lines.

1. Introduction

Red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae) are unique for their ability to biosynthesize a wide variety of secondary metabolites with diverse structural features depending on the species and localities.[1] The chemistry of halogenated compounds from *Laurencia* is a very interesting area of research and it never fails to offer the possibility of discovering new compounds with novel structures and properties.[2] The vast majority of *Laurencia* metabolites, so far, includes sesquiterpenes,[3,4] diterpenes,[5] triterpenes,[6] and C_{15}-acetogenins.[7] Most species of *Laurencia* biosynthesize a characteristic major metabolite or a class of compounds that are not commonly widely distributed within the genus.[8] Chemical studies based on cultured and field-collected materials of several species have revealed that the synthesis of halogenated secondary metabolites is not affected by environmental factors.[9] Thus, secondary metabolite chemistry can serve as an important tool for taxonomical studies in the genus *Laurencia* especially since many species appear morphologically very similar.[10] Moreover a number of halogenated metabolites have been shown to possess antibacterial, antifungal,[11] insecticidal[12] activities, as well as worth noting cytotoxicity against mammalian cells.[13]

During the course of our ongoing investigations toward the isolation and biological evaluation of compounds from marine organisms of the Greek seas,[14–16] we studied specimens of *Laurencia microcladia* Kützing, collected off the coasts of Chios island. In this report we describe the isolation and structure elucidation of five new metabolites (**1**–**4** and **8**) along with the known metabolites dibromophenol (**5**), (+)-α-isobromocuparene (**6**), and (−)-α-bromocuparene (**7**), all of which were obtained from the non-polar fractions of the organic extracts of *L. microcladia*.

An assessment of their cytotoxicity was performed on the following human tumor cell lines: HT29 (derived from colorectal adenocarcinoma), MCF7 (derived from a mammary adenocarcinoma), PC3 (derived from a prostate adenocarcinoma), HeLa (derived from cervix adenocarcinoma), and A431 (derived from epidermoid carcinoma).[17]

2. Results and discussion

L. microcladia was collected from the island of Chios and the CH_2Cl_2/MeOH extract of the freeze-dried alga was subjected to a series of vacuum column chromatography (VCC) on silica gel and normal phase high pressure liquid chromatography (HPLC), using mixtures of cyclohexane/EtOAc as the mobile phase, to yield compounds **1**–**8** in pure form.

Compound **1**, after HPLC purification, was isolated as colorless oil. The molecular formula $C_{15}H_{18}OBrI$ was deduced

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from HRFABMS data in combination with the NMR spectra (Tables 1 and 2). The LREIMS peaks at *m/z* 420/422 $[M]^+$, with relative intensities 1/0.9 $^{79}Br/^{81}Br$, indicated the presence of one bromine atom. The intense absorption at ν_{max} 3424 cm^{-1} showed the presence of a hydroxyl functionality in the molecule. The ^{13}C NMR and DEPT experiments allowed the determination of seven quaternary, two methine, three methylene, and three methyl carbon atoms. The ^1H and ^{13}C NMR spectra displayed resonances for one aromatic methyl ($\delta_{H/C}$ 2.61/29.8), two quaternary methyls ($\delta_{H/C}$ 1.28/18.7 and $\delta_{H/C}$ 1.37/22.4), one aromatic proton ($\delta_{H/C}$ 7.70/132.4), one methine ($\delta_{H/C}$ 1.09/24.2), cyclopropyl protons ($\delta_{H/C}$ 0.48, 0.52/16.2), and two methylenes ($\delta_{H/C}$ 1.61, 1.92/25.2 and $\delta_{H/C}$ 2.20, 1.19/35.1). All protonated carbons and their protons were assigned by the COSY and HMQC experiments. The structure elucidation was assisted by analyses of the HMBC experiments. The correlation in the HMBC experiments, between H-14 (δ_H 1.37) with C-1 (δ_C 49.1), C-5 (δ_C 35.1) and the aromatic carbon C-6 (δ_C 134.4) confirmed the position of H-14 on C-1. The correlation of signals at δ_H 1.28 (H-12) with 29.6 (C-2), 24.2 (C-3), and 16.2 (C-13) secured the position of H-12 on C-2. Comparison of the NMR data of **1** with reported values for laurinterol,[18,19] led to the assignment of the structure as the iodo-laurinterol. The shift of the aromatic methyl group (δ 2.61/29.8) in lower fields compared to laurinterol (δ 2.27/22.2) supported the position of the iodine on C-8. Furthermore, an HMBC correlation between H-11 with C-1 (δ_C 49.1) and C-6 (δ_C 134.4) confirmed the relative positions of the substituents on the aromatic ring. The strong NOE correlations between H-12/H-14 with H-5β and H-3 with H-4β determined the relative stereochemistry at C-1, C-2, and C-3. Consideration of the above data led to the determination of the structure as 8-iodo-laurinterol. As far as we know, this is only the third iodinated sesquiterpene from the genus *Laurencia*, besides 10-bromo-7-hydroxy-11-iodolaurene and a related iodo ether A that have been isolated from *Laurencia nana* Howe.[20]

Compound **2**, a bromo sesquiterpene ether, was purified by means of HPLC and was also isolated as colorless oil. Combination of its ^{13}C NMR data and HRFABMS measurements suggested a molecular formula of $C_{15}H_{17}OBr_3$. The LREIMS peaks at *m/z* 450/452/454/456 $[M]^+$, with relative intensities 1.0/3.0/2.9/0.9, indicated the presence of three bromine atoms. The IR absorption at 1240 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 **2** (Table 2) exhibited signals for 15 carbons with the multiplicities of the carbon signals determined from the DEPT spectrum as: seven quaternary, two methine, three methylene and three methyl carbon atoms. The ^1H and ^{13}C NMR spectra displayed resonances for one aromatic methyl ($\delta_{H/C}$ 2.50/23.2), one quaternary methyl ($\delta_{H/C}$ 1.35/20.1), one secondary methyl ($\delta_{H/C}$ 0.72/6.7), one aromatic proton ($\delta_{H/C}$ 7.20/126.9), and two protons on carbon bearing bromine at $\delta_{H/C}$ 3.52, 3.71/34.8. With six degrees of unsaturation, the structure was suggested to contain besides the aromatic ring, two other rings, one of which incorporates the ether linkage and the other a five-membered carbocyclic ring. The ^1H and ^{13}C NMR data of the compound **2** were similar to the reported values for the known metabolite bromoether A, isolated previously from

Table 1. ^1H NMR data (400 MHz) of compounds **1–3** and **5–8**

No	1	2	3	5	6	7	8
				^1H (δ)			
1	—	—	—	—	—	—	1.67 (s)
2	—	1.86 (m)	—	—	—	—	—
3	1.09 (ddd, 7.8, 4.9, 3.9)	—	1.09 (ddd, 7.7, 4.7, 4.0)	1.08 (ddd, 7.8, 4.9, 3.0)	4.44 (dd, 9.2, 9.2)	4.04 (dd, 9.9, 8.9)	5.31 (m)
4	1.61 (−α) (dd, 12.2, 8.3);	2.02 (−a) (m);	1.61 (−α) (m);	1.61 (−α) (dd, 12.0, 7.9); 1.92	2.18 (−a) (m); 2.50	2.18 (−a) (m);	2.03 (−a) (m);
	1.92 (−β) (dddd, 12.2, 11.7, 8.2, 4.4)	2.17 (−b) (m)	1.92 (−β) (m)	(−β) (dddd, 12.0, 12.0, 7.5, 4.1)	(−b) (m)	2.49 (−b) (m)	2.31 (−b) (m)
5	1.19 (−α) (m); 2.20 (−β) (dd, 13.7, 8.2)	1.69 (−a) (m)	1.23 (−α) (m); 2.17 (−β)	1.22 (−α) (m); 2.21 (−β)	1.58 (−a) (m); 2.69	1.94 (−a) (m)	—
		1.89 (−b) (m)	(dd, 13.5, 8.0)	(dd, 13.4, 7.5)	(−b) (m)	2.26 (−b) (m)	
6	—	—	—	—	—	—	1.72 (−a) (m); 1.82 (−b) (m)
7	—	—	—	—	7.25 (d, 7.2)	7.07 (s)	2.16 (m)
8	—	—	—	—	7.11 (d, 8.2)	7.07 (s)	—
9	—	—	—	—	—	—	4.86 (−a) (br s)
							5.13 (−b) (br s)
10	—	—	—	—	7.11 (d, 8.2)	7.07 (s)	2.12 (m)
11	7.70 (s)	7.20 (s)	7.80 (s)	7.68 (s)	7.25 (d, 7.2)	7.07 (s)	2.13 (m)
12	1.28 (s)	0.72 (d, 7.1)	1.31 (s)	1.28 (s)	0.63 (s)	0.59 (s)	5.13 (br s)
13	0.48 (−a) (dd, 7.8, 4.9);	3.52 (−a) (d, 10.4);	0.54 (m)	0.47 (dd, 7.8, 4.9); 0.51	1.08 (s)	1.06 (s)	—
	0.52 (−b) (dd, 4.9, 3.9)	3.71 (−b) (d, 10.4)		(dd, 4.9, 3.0)			
14	1.37 (s)	1.35 (s)	1.39 (s)	1.38 (s)	1.27 (s)	1.40 (s)	1.67 (s)
15	2.61 (s)	2.50 (s)	1.95 (s)	2.51 (s)	2.32 (s)	2.29 (s)	1.60 (s)
–OH	5.59 (s)		4.81 (s)	5.76 (s)			

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in parts per million. *J* values in parentheses are in hertz.