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A REARRANGED CHAMIGRENE DERIVATIVE AND ITS POTENTIAL BIOGENETIC PRECURSOR FROM A NEW SPECIES OF THE MARINE RED ALGAL GENUS *LAURENCIA* (RHODOMELACEAE)

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Key Word Index—*Laurencia*; Rhodomelaceae; Rhodophyta; marine algae; halogenated chamigrene; sesquiterpenoids.

Abstract—The structures of a novel rearranged sesquiterpenoid and a biogenetically-related chamigrene derivative have been determined by combined spectral and chemical methods. These sesquiterpenoids were components of an undescribed *Laurencia* species, and each was toxic toward the damselfish *Pomacentrus coeruleus*.

INTRODUCTION

Over the past decade, red seaweeds of the genus *Laurencia* have become of considerable interest based upon their unprecedented synthesis of a wide diversity of halogenated sesquiterpenes, diterpenes, acetogenins and aromatic compounds [1, 2]. In connection with our continuing investigations of the chemical adaptations of tropical marine algae, we recently encountered an apparently undescribed species of the red seaweed *Laurencia* along the east coast of central Florida. We wish to report that this *Laurencia* species produced halogenated sesquiterpenoids mainly of the chamigrene class. Described here, in detail, is the isolation and structure elucidation of two new sesquiterpenoids, **1** and **3**. The alcohol **1** possesses a new rearranged carbon skeleton thus adding further biosynthetic capacity to this interesting genus of marine algae. Alcohol **3**, a typical bromochamigrene, possesses the structural features required to act as a biosynthetic precursor to **1**.

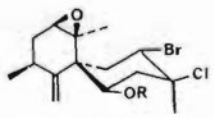
Although considerable chemical study has been directed toward the halogenated metabolites from *Laurencia* species, few studies have provided evidence of the biological significance of these molecules [3]. We believe haloterpenoid and haloacetogenin synthesis represents a defensive adaptation against the abundant herbivore populations in tropical habitats. Although still under investigation, we find the alcohols **1** and **3** to illustrate significant ichthyotoxicity toward reef fishes.

RESULTS AND DISCUSSION

The sesquiterpenoid alcohols **1** and **3** were isolated by conventional chromatographic methods from the $CHCl_3$–MeOH (2:1) extract of the alcohol preserved algae. Alcohol **1**, the major metabolite (5% organic extract) analysed for $C_{15}H_{22}O_2BrCl$ by its combined HRMS and ^{13}C NMR (Table 1) spectral features. Absorption at 3500 cm^{-1} in the IR spectrum of **1** indicated the compound was an alcohol. Indeed, treatment of **1** under standard acetylation conditions yielded the monoacetate **2**.

Consideration of the unsaturation inherent in **1**, taken in combination with ^{13}C NMR data, showed that the molecule was bicarbocyclic. The ^{13}C NMR spectrum showed the presence of an exomethylene functionality [154.8 (*s*), 104.1 (*t*)], a trisubstituted epoxide [61.0 (*s*), 59.4 (*d*)], a secondary, bromine-bearing carbon [64.8 (*d*)], a quaternary chlorine-bearing carbon [70.1 (*s*)] and a secondary alcohol carbon [68.7 (*d*)].

At 360 MHz the majority of the proton bands in **1** could be inter-related by spin-decoupling (Table 1). In C_6D_6 most resonances were even more resolved and complete assignments were made (Experimental). Three separate spin systems, C-1–C-2, C-4–C-5 and C-8–C-9–C-10 were readily discerned. The substituents, their coupling constants (characteristic of cyclohexane systems) and the presence of the spirocarbon at C-6 [^{13}C: 49.8 (*s*)] suggested that **1** possessed a spiro 5.5 undecane skeleton characterized in *Laurencia* species by the chamigrene skeleton [1]. Indeed, this was clearly consistent with the well-known ^{13}C NMR features for the bis-equatorial



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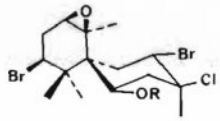
2 R = Ac



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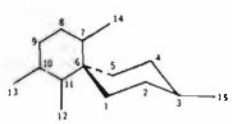

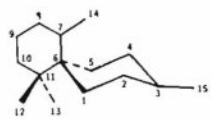

Table 1. ^1H and ^{13}C NMR assignments for sesquiterpenoids **1** and **3***

C No.	1			3		
	s m	*J* (Hz)	^{13}C†	*s m*	*J* (Hz)	^{13}C†
1	4.47 *dd*	12.6, 5.6	68.7 *d*	4.40 *dd*	13, 6.1	68.2 *d*
2	2.92 *m* 2.62 *dd*	11.2, 5.6	35.8 *t*	2.70 *m* 2.46 *dd*	13.4, 6.1	40.5 *t*
3	—		70.1 *s*	—		70.3 *s*
4	4.71 *dd*	13.5, 4.2	64.8 *d*	4.87 *dd*	13.2, 5.5	64.0 *d*
5	2.95 *m* 1.54 *m*		46.3 *t*	2.70 *m* 2.12 *dd*	13.9, 13.3	49.4 *t*
6	—		49.8 *s*	—		50.6 *s*
7	—		61.0 *s*	—		62.4 *s*
8	2.95 *m*		59.4 *d*	2.92 *m*		61.0 *d*
9	1.54 *m* 1.22 *m*		43.0 *t*	2.54 *m* 2.54 *m*		39.1 *t*
10	2.35 *m*		27.1 *d*	4.96 *dd*	9.4, 8.8	61.9 *d*
11	—		154.8 *s*	—		44.1 *s*
12	5.00 *s* 4.89 *s*		104.1 *t*	1.18 *s*		19.8 *q*
13	1.03 *s*		18.4 *q*	1.16 *s*		25.6 *q*‡
14	1.69 *s*		25.5 *q*‡	1.58 *s*		28.3 *q*‡
15	1.77 *s*		27.1 *q*‡	1.75 *s*		29.1 *q*‡

*^1H assignments were made by spin-decoupling and ^{13}C assignments were made by comparison of the data with suitable chamigrene models. ^1H spectra were recorded in $CDCl_3$ solution at 360 MHz with internal TMS as standard; ^{13}C spectra were recorded in bz-d_6 solution at 50 MHz with TMS as internal standard; abbreviations: *m* = multiplet, *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet.

†Multiplicities determined by single frequency off-resonance decoupling techniques.

‡Values may be interchanged.

dihalide constellation commonly encountered in *Laurencia* chamigrenes [4]. However, decoupling analysis clearly showed that the usual C-10 bromine component of halochamigrenes was absent. Instead, it could clearly be seen, in C_6D_6 solution, that an axial proton was present, coupled to a methylene pair at C-9 (J = 13.9, 1.5 Hz) but also to an equatorial methyl group (J = 6.6 Hz). This same proton sharpened when the exomethylene proton at δ4.56 was irradiated, thus placing the exomethylene component at C-11.

The relative stereochemistries of the substituents, C-1, C-3, C-4 and C-7, C-8 were assigned based upon coupling constant analyses for 6-membered rings and upon NOE measurements. The alcohol at C-1 and the bromine at C-4 were clearly equatorial since the corresponding protons showed large axial–axial couplings. Fortuitously, a NOE experiment successfully related the epoxide stereochemistry to the overall configuration of the vicinal dihalide-bearing ring. Irradiation of the epoxide methyl group at δ 1.69 resulted in the expected enhancement of the epoxide proton at δ 2.95, but also enhanced the axial proton on the adjacent ring at C-4. This latter enhancement can only occur if the C-7 epoxide methyl (C-14) and the C-5–C-4 substituent at C-6 are both 'down' as arranged in structure **1**. This experiment was fortuitous in that it successfully related the relative stereochemistries of the two carbon rings.

The structure of alcohol **1** was thus assigned as the rearranged chamigrene resulting, biogenetically, from an apparent methyl migration from C-11 to C-10. Rearrangements of this nature, fostered by solvolysis of the bromine atom at C-10, have been implicated in the biosynthesis of numerous rearranged carbon skeletons isolated from *Laurencia* species [5]. Alcohol **1**, however, represents the first example of this rearrangement within the chamigrene class of sesquiterpenoids.

The unrearranged chamigrene alcohol **3** was also isolated from this extract but as a more minor component (2% organic extract). Alcohol **3** analysed for $C_{15}H_{23}O_2Br_2Cl$ by HRMS and ^{13}C NMR. The spectral features of **3** were similar to **1** and highly analogous to several closely related *Laurencia* chamigrenes [6]. As in **1**, acetylation yielded the monoacetate **4**, and complete proton spin-decoupling analyses led to the complete assignments listed in the Table. The complete structure assignment of alcohol **3** was thus based upon the complete analogy of the spectral data of **3** with several closely related chamigrenes [6]. No efforts were made to determine the absolute stereochemistries of these metabolites.

In initial experiments designed to predict the biological functions of *Laurencia* metabolites the ichthyotoxicities of alcohols **1** and **3** were measured using methods already described [7]. At concentrations of 15 μg/ml alcohols **1** and **3** were lethal to the damselfish *Pomacentrus coeruleus* within a 1 hr period.

EXPERIMENTAL

Extraction and isolation procedures. Laurencia species, Smithsonian herbarium No. JN-11540, was collected August 19, 1982 at Rio Mar near Vero Beach, Florida. The Florida plants resemble *L. flagellifera* as reported from Brazil. However, J.N. considers this may represent an undescribed species until further anatomical studies can be done comparing Florida and Brazil specimens. The algae were immediately preserved in EtOH solution, and next thoroughly extracted with $CHCl_3$–MeOH (2:1) to yield 2.5 g crude condensed extract. The extract was chromatographed over silica gel using conventional methods and several fractions were combined (eluted with 20% EtOAc in isooctane) and further purified by silica preparative HPLC (same solvent). These procedures resulted in the isolation of alcohols **1** (125 mg, 5% ext.) and **3** (50 mg, 2% ext.) as light mobile oils.

Rearranged chamigrene **1** *and acetate* **2**. The rearranged chamigrene alcohol **1** showed $[\alpha]_D$ +67° (*c* 0.5, $CHCl_3$) and analyzed for $C_{15}H_{22}O_2BrCl$ by HRMS, for $[M-OH]^+$ calc.: 331.0462, obsd. 331.0451; for $[M-HCl]^+$ calc.: 312.0724, obsd. 312.0699. The alcohol showed the following spectral features not reported in the text Table 1: IR ($CHCl_3$): 3500, 2950, 1620, 1450, 1375, 1100, 1040 cm^{-1}; ^1H NMR (360 MHz, C_6D_6): δ 3.98, 1H, *dd*, *J* = 13.0, 5.7 (C-1); 2.89 1H, *t*, *J* = 13.0 (C-2); 2.37, 1H, *dd*, *J* = 13.0, 5.7 (C-2'); 4.91, 1H, *dd*, *J* = 13.5, 4.2 Hz (C-4); 2.51, 1H, *bs* (C-8); 1.84, 1H, *m* (C-9); 1.00, 1H, *ddd*, *J* = 13.9, 13.9, 1.5 Hz (C-9'); 2.06, 1H, *ddd*, *J* = 13.9, 6.6, 1.5 Hz (C-10); 4.56, 1H, *d*, *J* = 1.1 Hz (C-12); 4.54, 1H, *s* (C-12'); 0.73, 3H, *d*, *J* = 6.6 Hz (C-13); 1.52, 3H, *s* (C-14); 1.56, 3H, *s* (C-15). *Acetate* **2**. Acetylation of **1** (5 mg) was performed with a 20% molar excess of acetic anhydride in pyridine at room temp. for 24 hr. Ether extraction of the reaction mixture followed by water, acid and $NaHCO_3$ wash of the combined ether extracts and reduction *in vacuo* yielded the monoacetate **2** which was not further purified. Acetate **2** showed the following spectral features: $[\alpha]_D^{25}$ +61° (*c* 0.6, $CHCl_3$); HRMS for $[M-CHO]^+$ calc. 361.0568, obsd. 361.0536; IR ($CHCl_3$): 2960, 1730, 1450, 1360, 1260 cm^{-1}. ^1H NMR (360 MHz, $CDCl_3$): δ 5.50 (1H, *dd*, *J* = 10.7, 7.2 Hz), 4.72 (1H, *s*), 4.71 (1H, *s*), 3.06 (1H, *dd*, *J* = 14.1, 4.1 Hz), 2.98 (1H, *s*), 2.76 (2H, mult), 2.30 (1H, mult), 2.23 (1H, mult), 2.05 (3H, *s*), 1.81 (3H, *s*), 1.67 (3H, *s*), 1.60 (2H, mult), 1.41 (1H, *t*, *J* = 13 Hz), 1.00 (3H, *d*, *J* = 6.6 Hz).

Chamigrene alcohol **3** *and acetate* **4**. The chamigrene alcohol **3** showed $[\alpha]_D$ +8.8° (*c* 1.5, $CHCl_3$) and analyzed for $C_{15}H_{23}O_2Br_2Cl$ by HRMS calc for $[M-Br]^+$ 349.0570 obsd: 349.0573. Alcohol **3** showed the following IR absorptions ($CHCl_3$): 3500, 2960, 1450, 1390, and 1210 cm^{-1}. *Acetate* **4**. The alcohol **3** (5 mg) was acetylated in a fashion identical as with **1** to yield the monoacetate **4** which was not purified further. The acetate showed the following spectral features: IR ($CHCl_3$): 2950, 1730, 1450, 1360, 1260 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.47 (1H, *dd*, *J* = 12.1, 6.1 Hz), 4.83 (1H, *dd*, *J* = 13.2, 5.4 Hz), 4.14 (1H, *dd*, *J* = 10.8, 7.7 Hz), 2.94 (1H, *d*, *J* = 4.6 Hz), 2.69 (2H, mult), 2.54 (3H, mult), 2.11 (3H, *s*), 1.79 (3H, *s*), 1.60 (3H, *s*), 1.18 (3H, *s*), 1.15 (3H, *s*).

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