PERGAMON

Phytochemistry 50 (1999) 745–748

PHYTOCHEMISTRY

Sesquiterpenes from *Laurencia claviformis*

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Received 31 March 1998; accepted 1 September 1998

Abstract

A new halogenated sesquiterpene has been isolated, together with five known compounds, from the red alga *Laurencia claviformis*, a species endemic to Easter Island. The structures were elucidated based on spectral analysis. The effect of these compounds on the inhibition of cytokinesis in the sea urchin *Tetrapygus niger* embryos was studied.

Keywords: Laurencia claviformis; Rhodomelaceae; Red alga; Halogenated metabolites; Sesquiterpenes; Easter Island

1. Introduction

Laurencia is the most studied genus of all marine algal genera by natural product researchers. It has been established that this genus is an extremely rich source of secondary metabolites, predominantly in two major biosynthetic halogenated groups: terpenes (sesquiterpenes, diterpenes and triterpenes) and C_{15}-acetogenins (Faulkner, 1997; and earlier reviews in this series). Non-halogenated metabolites have also been isolated from some *Laurencia* species (San-Martín, Rovirosa, Xu, Lu, & Clardy, 1987). The species discrimination in the red algal genus *Laurencia* is complicated by a high degree of morphological variation within individual species (Masuda, Abe, Suzuki, & Suzuki, 1996). As diverse halogenated secondary metabolites are characteristic in *Laurencia*, in the past they were considered as useful taxonomic markers at the species level (Fenical and Norris, 1975).

In previous studies we found that the red alga *L. claviformis* from Easter Island is a rich source of halogenated sesquiterpenes and acetogenins (Rovirosa, Astudillo, Sánchez, Palacios, & San-Martín, 1989; San-Martín et al., 1997). Pacifenol, the major compound, had been used as precursor in the synthesis of several plaguicide derivatives (Rovirosa et al., 1994). This sesquiterpene is produced from prepacifenol by acid catalysis (Sims, Fenical, Wing, & Radlick, 1973). In our continuing search for bioactive compounds from marine sources (Rovirosa and San-Martín, 1997), and in order to determine the effects of *L. claviformis* metabolites on the inhibition of cytokinesis in sea urchin *Tetrapygus niger* embryos, a further detailed investigation of the minor metabolites of this alga was undertaken. We report here on the structure determination of claviol, a new compound, together with five known sesquiterpenes with the chamigrene skeleton, and their biological activity.

2. Results and discussion

The organic extract of *L. claviformis* on chromatographic purification on Sephadex LH-20 and silica gel columns yielded a new halogenated sesquiterpene and several known analogs. Compound **1** was isolated as a stable colorless and optically active, $[\alpha]^{25}_{D}$, $+6.8^{\circ}$ (c 0.97, $CHCl_3$), crystalline solid (mp 138°C) with a molecular formula of $C_{15}H_{21}O_2Br$ as evidenced by NMR spectroscopy and the HREIMS. The ^{13}C NMR decoupled spectrum of **1** (Table 1) showed well-resolved resonances for all 15 carbons. DEPT 90° analysis indicated two sp^2 methine carbons at δ 122.3 and 131.7 and two saturated methines at δ 81.0 and 82.7. The DEPT 135° spectrum showed two methylene and four methyl carbons indicating, after comparison with the decoupled spectrum, that the carbons at δ 49.3, 51.3, 79.9, 133.2 and 142.6 were nonhydrogenated.

The ^1H NMR spectrum of **1** showed two vinyl proton signals at δ 6.4 (1H, d, $J=6.5$ Hz) and 5.43 (1H, m). One vinylic methyl signal at δ 1.70 (3H, s), together with four

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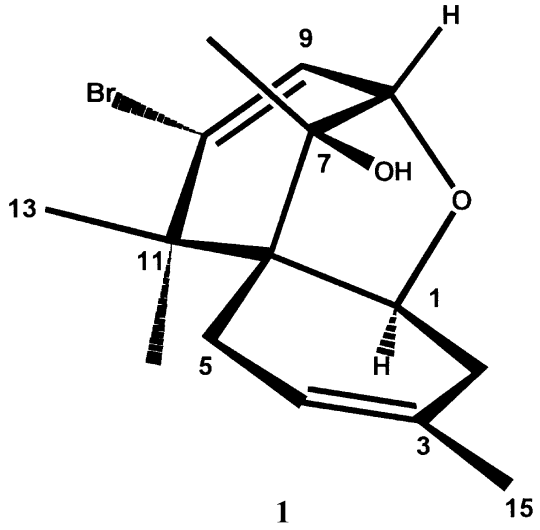
0031-9422/99/$ - see front matter
PII: S0031-9422(98)00617-7

Table 1
^{1}H and ^{13}C NMR data for compound **1**[a]

Position	^{1}H NMR (400 MHz, $CDCl_3$)	^{13}C NMR (100 MHz, $CDCl_3$)	DEPT multiplicity	HMBC
1	4.85 (1H,dd, $J=7.0$, 12.6 Hz)	82.7	CH	H-2, H-8
2	α: 2.59 (1H, bd, $J=12.6$ Hz) β: 2.35 (1H, m)	34.9	CH_2	H-1, H-15
3	–	133.2	C	H-2, H-15
4	5.43 (1H, m)	122.3	CH	H-15
5	α: 2.51 (1H, bd, $J=7.0$ Hz) β: 2.33 (1H, m)	26.0	CH_2	
6	–	51.3	C	H-5, H-8, H-12, H-13, H-14
7	–	79.9	C	H-8, H-9, H-14
8	3.83 (1H, d, $J=6.5$ Hz)	81.0	CH	H-14
9	6.40 (1H, d, $J=6.5$ Hz)	131.7	CH	
10	–	142.6	C	H-8, H-9, H-12, H-13
11	–	49.3	C	H-1, H-9, H-12, H-13
12	1.27 (3H, s)	27.3	CH_3	H-13
13	1.47 (3H, s)	25.9	CH_3	
14	1.49 (3H, s)	23.5	CH_3	
15	1.70 (3H, s)	23.2	CH_3	

[a]All assignments are based on extensive 1-D and 2-D NMR experiments, including COSY and HMQC.



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signals in the ^{13}C NMR spectra belonging to sp^2 carbons, established the presence of two double bonds. The hydroxyl group was confirmed by the band at 3350 cm^{-1} in the IR spectrum, and it was assigned as a tertiary carbon on the basis of its NMR data: δ_H 1.49 (3H, s) and δ_{13C} 23.5 (CH_3) and 79.9 (C). The doublet at δ 3.83 (1H, d, $J=6.5$ Hz) and the double doublet at δ 4.85 (1H, dd, $J=7.0$, 12.6 Hz) observed in the ^{1}H NMR spectrum corresponded to methine protons geminal to an oxygenated functionality. The ^{1}H–^{1}H COSY spectrum revealed that the latter signal was coupled to a methylene which appeared at δ 2.35 (1H, m) and 2.59 (1H,bd, $J=12.6$ Hz). This spectrum also showed that the vinylic protons (δ 5.43 and δ 6.40) were coupled to a methylene group (δ 2.33 (1H, m) and 2.51 (1H, bd, $J=7.0$ Hz)) and to a methine at δ 3.83 (1H,d, $J=6.5$), respectively. The J value of H-1 ($J=12.6$ Hz) suggests a *trans* diaxial disposition with one of the protons of the adjacent methylene and it is similar to the corresponding proton in pacifenol 2 and its dehydrochlorine derivative (Darias, San-Martín, & Rovirosa, 1990), suggesting the same relative stereochemistry at C-1. Detailed analysis of the ^{1}H and ^{13}C spectra, plus ^{1}H–^{1}H, COSY, HMQC, ROESY and HMBC experiments, allowed the unambiguous assignment of the ^{1}H and ^{13}C signals shown in Table 1. HMQC and HMBC data were used to confirm linkage C-1/C-8 which was established by the correlation between C-1 and H-8. The relative stereochemistry of the chiral centers of the rings were determined by ROESY experiments, which in the 2-D-ROESY experiment showed NOE between H-1 and H-12 and H-2 (δ 2.33). The same effect was observed between H-8 and H-9 and H-14. From the above data, it was evident that claviol had the structure depicted in **1**.

Biogenetically, claviol can be considered a derivative of 3,4-dehaloprepacifenol 7 by opening of the epoxide by S_N2 attack of the hydroxyl group at C-1. This way of internal cyclization, unlike pacifenol which is derived from prepacifenol via a S_N2'-type rearrangement (Darias et al., 1990) following the sequence (7→3→2), can be associated with the absence of halogen substituents in ring B of the precursor 7. Claviol presents a new skeleton

derived from dehaloprepacifenol cyclization. Other known terpenoids, pacifenol **2**, prepacifenol **3**, deoxyprepacifenol **4**, 9-hydroxy-4,10-dibromo-3-chloro-α-chamigrene **5** and 4,10-dibromo-3-chloro-α-chamigrene **6** were also isolated (see Section 3).

In the inhibition of fertilized sea urchin assay (Table 2), terpenoid **5** was more active. However, this activity could be considered mild compared with other active marine compounds as stypoldione, which show an $ED_{50} = 1.1$ μg/mL (O'Brien, Asai, Jacobs, & Wilson, 1989). This test appears to be a reasonable prescreen, nevertheless, to determine which substances merit further evaluation for antineoplastic properties (Munro, Luibrand, & Blunt, 1987).

3. Experimental

3.1. General

Melting points were determined on a Kofler block and are uncorrected. The solvents used for spectral measurements were TMS-$CDCl_3$. ^1H and ^{13}C NMR spectra, as well as ^1H–^1H COSY, DEPT, HMBC and HMQC (optimized for $^1J_{H-C} = 140$ Hz) and ROESY (mixing time of 250 ms) data were obtained on Bruker AMX-300 and AMX-400 spectrometers. IR spectrum and specific rotations were determined on a spectrophotometer FT-IR-Raman Perkin-Elmer System 2000 and a Perkin-Elmer 241 polarimeter, respectively. Mass spectra were determined on a Hewlett Packard 5995 and VG Micromass ZAB-2F mass spectrometers. Column chromatography was carried out on silica gel (70–230 mesh Merck); TLC was performed on precoated Kieselgel 60F-254 plates 0.5 mm thick (Merck). Spots were visualized under UV light (254 nm), irradiation and by spraying with 10% H_2S0_4 solution followed by heating. Anhydrous sodium sulfate was used for drying solutions

3.2. Collection

L. claviformis Börgensen 1924 was collected at low tide pools at Vaihú Easter Island, Chile, in November 1996. A voucher specimen is deposited in the Herbarium, Museo de Historia Natural de Santiago, Chile (No. V-96/2) and Professor Ramírez authenticated the alga identification.

Table 2
Inhibition of cytokinesis in the sea urchin *T. niger*

Compound	ED_{50}
1	69.5
2	45.2
3	>150
4	>150
5	22.7
6	50.5

3.3. Extraction and isolation

The fresh alga was drained of excess water and frozen immediately. After approximately one month, the alga was thawed and homogenized in a blender. The solid was removed (1150 g dry weight) by filtration and extracted with *n*-hexane and chloroform. The organic filtrate was separated from the aqueous phase and dried over sodium sulfate. The organic extracts were combined and the solvents removed to obtain a viscous oil (54 g). The extract was chromatographed over Sephadex LH-20 using 2 L of a mixture of *n*-hexane–chloroform–methanol (7:2:1) as eluent. In this way the major compound, pacifenol 2 (4.2 g) was isolated. Fractions exhibiting similar TLC profiles were combined. A portion (8.5 g) of the combined fractions was chromatographed on silica gel cc using a petroleum ether–EtOAc solvent mixture of increasing polarity as eluent. Further separations over silica gel on a Chromatotron with *n*-hexane–EtOAc (8.5:1.5) yielded the following compounds previously isolated from the same alga (Rovirosa, Astudillo, Sánchez, Palacios, & San-Martín, 1989): prepacifenol **3** (190 mg), deoxyprepacifenol **4** (95 mg), 9-hydroxy-4,10-dibromo-3-chloro-α-chamigrene **5** (50 mg) and 4,10-dibromo-3-chloro-α-chamigrene **6** (70 mg) (Howard and Fenical, 1975). Claviol (30 mg) crystallized from *n*-hexane.

3.4. Claviol *1*

White crystals; mp 138°C (*n*-hexane); $[\propto]^{25}_D + 6.8°$ (c 0.97, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3350, 2900, 1650, 1380, 1120, 910 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HREIMS observed *m*/*z* 312.063 (**1**) (calcd. for $C_{15}H_{21}O_2\ ^{79}Br$) required 312.072 EIMS *m*/*z* 294/296 (2/2) (M^+–H_2O), 233 (46) (M^+–Br), 215 (37) (M^+–Br–H_2O), 178 (98), 176 (95).

3.5. Inhibition of cytokinesis in the sea urchin Tetrapygus niger

Drug incubation eggs and sperm were obtained from the sea urchin *T. niger* by intracoelomic injection of 0.5 M KCl. Eggs were shed into ice-cold, charcoal filtered, sea water and washed by three cycles of settling and resuspension in fresh filtered sea water. Sperm were collected undiluted and stored on ice.

Eggs were fertilized by adding sperm to a small volume of settled eggs. When fertilization envelops became visible by bright-field microscopy, approximately 20–30 s after fertilization, embryos were diluted into seawater to make a 1 vol% solution of eggs in seawater and were gently

sedimented. Eggs were resuspended in the seawater and resedimented twice more to remove excess sperm. The compounds and the vehicle control were dissolved in DMSO (1 mL). The final concentration desired was obtained using a fraction of the DMSO solution and adding 0.1 mL Tween 80 solution 1% and sea water to complete to 9.0 mL. This volume was added to 1 mL of the embryo suspension, 15 min after fertilization, with gentle mixing, to produce the desired final drug concentration (or solvent control) on a 10 mL volume total. At 15°C and under the incubation conditions used, the first cytokinesis was complete at approximately 120 min and the second taking place at 170–180 min. The effects of each treatment were evaluated in 100–120 embryos, which were examined microscopically at a slide with a drop of suspension on it. Unfertilized eggs were not counted. This test was applied at 3 different concentrations (μg/mL) and replicated three times. ED_{50} values were calculated by the method of Finney (1971).

Acknowledgements

This work was supported by grants from the Universidad de Chile (DTI, Q-3234/9645), Fondecyt No. 1950688 and Proyecto de Investigación conjunta de la Subdirección General de Cooperación Internacional de España.

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