Halogenated Helianane Derivatives from the Sponge *Spirastrella hartmani*

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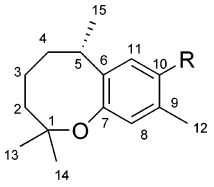
Two new halogenated derivatives (**1** and **2**) of helianane (**3**) were isolated from the 2-propanol extract of the sponge *Spirastrella hartmani*. The structures of the new derivatives were determined on the basis of detailed spectroscopic analysis, including (+)-HREIMS and 1D and 2D NMR. Compound **1** showed in vitro cytotoxicity against the human tumor cell lines A549, HT29, and MDA-MB-231.

Aromatic bisabolene sesquiterpenes have been found in a number of marine organisms including the sponges *Epipolasis* sp.,[1] *Didiscus flavus*,[2] *Arenochalina* sp.,[3] *Haliclona fascigera*,[4] and *Parahigginsia* sp.[5] and the gorgonian coral *Pseudopterogorgia rigida*.[6] Several compounds of this family display biological activities, the most noteworthy being the antifungal (*Candida albicans*), cytotoxic, and H/K-ATPase inhibitory activity of (−)-curcuphenol. More recently, cytotoxic activity has been described for parahigginols B−D and parahigginic acid.[5]

Helianane (**3**) is the only compound possessing both an aromatic bisabolene skeleton and a heterocyclic ring described from marine sources.[4] Herein we report the isolation of two additional members of the bisabolene class possessing this structural feature.

As part of our continuous screening program in search of new marine anticancer agents, we have focused our attention on the hitherto uninvestigated sponge *Spirastrella hartmani* Boury-Esnault et al. (1999) (phylum Porifera, family Spirastrellidae, order Hadromerida, class Demospongiae) due to the cytotoxicity displayed by its organic extract. Bioassay-guided fractionation of this extract afforded helianane **3** and two new halogenated derivatives (**1** and **2**).



1 R=Cl
2 R=Br
3 R=H

The 2-propanol extract of a frozen specimen of *S. hartmani* collected in Martinique was subjected to reversed-phase VLC on Lichroprep RP-18 silica gel and semi-preparative HPLC to yield compounds **1**−**3**. Helianane **3** was identified by comparison of its spectroscopic features with those reported.[4]

(+)-HREIMS ($[M]^+$ *m/z* 252.1282) and ^{13}C NMR spectra supported a molecular formula of $C_{15}H_{21}ClO$ for compound **1**. A comparison between the ^{13}C NMR chemical shifts of compound **1** and those reported for **3** revealed a closely related structure for both compounds. The major difference was the presence of a chlorine atom in the structure of **1**. This chlorine atom was located at the C-10 position of the aromatic ring on the basis of the following considerations. Signals for only two aromatic protons (H-8 br s and H-11 s) were observed in the ^1H NMR spectrum of **1**. The *p*-disposition of both protons was deduced from the absence of coupling and from the correlations in the HMBC spectrum between H-11 and C-5, C-7, C-9, and C-10 and between H-8 and C-6, C-7, C-10, and C-12. The slight differences in the chemical shifts of the aromatic ring in the ^{13}C NMR spectrum of **1** with respect to **3** are also in agreement with the location of the chlorine atom at C-10. Chemical shifts in the ^1H and ^{13}C NMR spectra for the nonaromatic moiety are almost identical to those reported for helianane **3** (Table 1).[4] The structure of the compound was confirmed by 2D NMR spectroscopy. According to their common biogenetic origin, the configuration of this sesquiterpene at C-5 was proposed as *S* by analogy with that reported for **3**. The positive value observed for the optical rotation of **1** is in agreement with the pattern found for other aromatic bisabolene derivatives having C-5 as the only chiral center and further supports the configuration proposed.[4]

Table 1. NMR Data for Compounds **1** and **2**, in CD_3OD

	1		**2**	
no.	δ_C mult	δ_H mult (*J* in Hz)	δ_C mult	δ_H mult (*J* in Hz)
1	71.5 C		71.5 C	
2	44.8 CH_2	1.43 m 2H	44.8 CH_2	1.43 m 2H
3	23.4 CH_2	1.30 m 2H	23.4 CH_2	1.30 m 2H
4	38.8 CH_2	1.49 m	38.8 CH_2	1.49 m
		1.60 m		1.61 m
5	33.0 CH	3.10 m	33.0 CH	3.11 m
6	134.5 C		135.4 C	
7	154.7 C		155.4 C	
8	118.3 CH	6.64 br s	118.3 CH	6.68 s
9	134.4 C		134.9 C	
10	125.2 C		114.7 C	
11	128.1 CH	7.00 s	131.4 CH	7.18 s
12	19.7 CH_3	2.22 s	22.6 CH_3	2.26 s
13	29.2 CH_3	1.12 s	29.3 CH_3	1.14 s
14	29.0 CH_3	1.11 s	29.0 CH_3	1.13 s
15	21.4 CH_3	1.16 d (6.5)	21.4 CH_3	1.18 d (7.0)

The ^1H NMR spectrum of **2** was nearly identical to that of **1**. The major differences were found in the chemical shifts of the aromatic protons. All the spectroscopic features of **2** were in agreement with the replacement of the chlorine

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atom at C-10 in **1** by bromine. Indeed, a molecular peak in the (+)-HREIMS ($[M]^+$ *m/z* 296.0775) with an isotopic pattern characteristic for bromine and the presence of 15 signals in the ^{13}C NMR spectrum accounted for a molecular formula of $C_{15}H_{21}BrO$. The location of the bromine atom at C-10 was supported by the differences in chemical shifts observed for carbons of the aromatic ring in **2** with respect to **1** (Table 1). Carbon C-10 was upfield shifted by 10.5 ppm in **2**, whereas no significative differences were found for the rest of the carbons in the ring. The deshielding of H-11 from δ_H 7.00 in **1** to δ_H 7.18 in **2** also confirms the location of the bromine atom at C-10. As for compound **1**, and on the basis of biogenetic considerations and the positive value obtained for its optical rotation, the absolute configuration at C-5 in **2** is proposed as *S*.

The cytotoxic activity of compounds **1** and **2** was tested against three human tumor cell lines (A549, HT29, and MDA-MB-231). Compound **1** exhibited mild activity with GI_{50} values of 37.2 (A549), 37.6 (HT29), and 37.6 μM (MDA-MB-231), whereas compound **2** was inactive against the three lines tested. GI_{50} values obtained for a positive control tested under the same conditions (Yondelis) were 26.3 (A549), 19.7 (HT29), and 18.4 nM (MDA-MB-231).

Experimental Section

General Experimental Procedures. Optical rotations were determined in $CHCl_3$ using a Jasco P-1020 polarimeter. UV spectra were obtained with a Perkin-Elmer Lambda 15 UV/vis spectrophotometer. IR spectra were recorded on a Perkin-Elmer 881 infrared spectrophotometer. NMR spectra were recorded on a Varian "Unity 500" spectrometer at 500/125 MHz ($^1H/^{13}C$). Chemical shifts were reported in ppm using residual CD_3OD (δ 3.34 for ^1H and 49.0 for ^{13}C) as internal reference. (+)-HREIMS was performed on a VGAutoSpec spectrometer.

Animal Material. *Spirastrella hartmani* was collected in April 2003 by scuba diving at a depth of 10−20 m in Tunel du Diamant, Martinique. The material was identified by Dr. José Luis Carballo (UNAM, México). A voucher specimen (ORMA025536) is deposited at PharmaMar.

Extraction and Isolation. The frozen sponge (135 g) was diced and exhaustively extracted with 2-propanol (3 × 500 mL). The extract was concentrated under vacuum to yield a crude of 4.06 g. This crude material was subjected to VLC on RP-18 silica gel with a stepped gradient from H_2O to MeOH. Fractions eluted with MeOH were subjected to semipreparative HPLC (Symmetry C18, 19 × 150 mm, gradient H_2O/CH_3CN from 40 to 65% CH_3CN in 20 min, 15 mL/min, UV detection) to yield three fractions (F1−F3). Fraction F1 was further purified by semipreparative HPLC (Symmetry C18, 7.8 × 150 mm, gradient H_2O/CH_3CN from 40 to 65% CH_3CN in 20 min, 2.5 mL/min, UV detection) to yield 1.0 mg of pure helianane **3**. Compound **1** (3.9 mg) was isolated from fraction F2 by semipreparative HPLC (Symmetry C18, 7.8 × 150 mm, gradient H_2O/CH_3CN from 40 to 65% CH_3CN in 40 min, 2.5 mL/min, UV detection). Semipreparative HPLC (Spherisorb S5 ODS-2, 10 × 250 mm, gradient H_2O/CH_3CN from 40 to 65% CH_3CN in 20 min, 2.5 mL/min, UV detection) of fraction F3 yielded compound **2** (1.9 mg).

Compound 1: pale yellow oil; $[\alpha]^{25}_D$ +80.0 (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} 230, 285 nm; IR (KBr) ν_{max} 2965, 1440, 1411, 1258, 1014 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; (+)-HREIMS *m/z* $[M]^+$ 252.1282 (calcd for $C_{15}H_{21}{}^{35}ClO$ 252.1281, Δ 0.1 mmu).

Compound 2: pale yellow oil; $[\alpha]^{25}_D$ +2.8 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} 230, 289 nm; IR (KBr) ν_{max} 2965, 2853, 1606, 1451, 1395, 1258, 1092 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; (+)-HREIMS *m/z* $[M]^+$ 296.0775 (calcd for $C_{15}H_{21}{}^{79}BrO$ 296.0776, Δ −0.1 mmu).

Biological Activity. A colorimetric type of assay using sulforhodamine B reaction has been adapted for a quantitative measurement of cell growth and viability following the technique described.[7] The in vitro activity of the compounds was evaluated against three human tumor cell lines, including NSCL (A549), colon (HT29), and breast (MDA-MB-231).

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