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J. Nat. Prod., **1991**, 54 (4), 1108-1111• DOI:
10.1021/np50076a032 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 1108-1111, Jul-Aug 1991

AN ANTIVIRAL SESQUITERPENE HYDROQUINONE FROM THE MARINE SPONGE *STRONGYLOPHORA HARTMANI*

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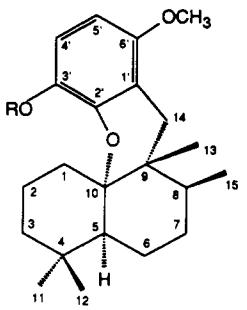
ABSTRACT.—A new sesquiterpene hydroquinone **1**, which we call strongylin A, was isolated from the marine sponge *Strongylophora hartmani*. Its structure was determined through spectroscopic methods, including 2D-^{13}C homonuclear correlation spectroscopy (INADEQUATE). Both **1** and its acetate derivative are active in in vitro assays against the P-388 tumor cell line and influenza strain PR-8.

Strongylophora hartmani van Soest (Haplosclerida) (1) is a stony marine sponge that occurs in deep water habitats throughout the Caribbean. An earlier investigation of *S. hartmani* carried out in this laboratory yielded puupehenone as the major cytotoxic component (2). Reinvestigation of a different sample of *S. hartmani*, to determine if additional biologically active agents were present, yielded a new sesquiterpene hydroquinone **1**, which we have called strongylin A. Puupehenone was also present in this sample (0.14% wet wt). Compound **1** inhibits growth of the P-388 murine leukemia tumor cell line and the influenza strain PR-8. This paper describes the isolation and structure elucidation of **1**.

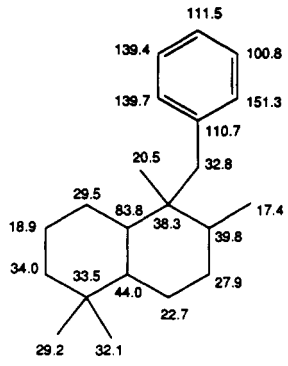
A sample of *S. hartmani* was collected at a depth of 345 m using a Johnson-SEA LINK submersible off Wood Cay, Grand Bahama Island, Bahamas and frozen immediately for transport to the laboratory. An EtOAc extract of the sponge (209 g) was chromatographed repeatedly on Si gel to yield 620 mg of **1** (0.29% wet wt).



1 R=H
2 R=Ac

High resolution eims of **1** suggested a molecular formula of $C_{22}H_{32}O_3$ (*m/z* calcd 344.2353, found 344.2357) requiring seven sites of unsaturation. Six olefinic carbons were observed in the ^{13}C-nmr spectrum of **1**, accounting for three of the sites of unsaturation. Lack of evidence for further unsaturation suggested that **1** has four rings. The ^{1}H-nmr spectrum indicated the presence of two coupled aromatic protons [δ 6.91 (d, J = 8.6 Hz), 6.13 (d, J = 8.6 Hz)], one D_2O exchangeable proton (δ 5.12, s), one methoxy group (δ 3.44, s, 3H), one methyl doublet (δ 0.90, d, J = 7.5 Hz, 3H), three methyl singlets [δ 1.09 (s, 3H), 0.82 (s, 3H), 0.65 (s, 3H)], and an isolated benzylic methylene group [δ 3.20 (d, J = 17.6 Hz), 2.30 (d, J = 17.6 Hz)]. A resonance observed at 83.8 ppm in the ^{13}C-nmr spectrum suggested the presence of an ether in **1**. Acetylation of **1** with Ac_2O in pyridine gave the monoacetate **2**, confirming the presence of phenolic functionality in **1**. The spectral data for **1** was reminiscent of that reported for the terpene hydriquinone aureol (3), suggesting that **1** belongs to this class of compounds.

As large quantities of **1** were available for nmr analysis, the carbon skeleton **3** was derived from interpretation of two 2D ^{13}C homonuclear correlation experiments (INADEQUATE) (4,5). All of the carbon-carbon correlations which



3

define partial structure **3** were observed. As the correlations which define the aromatic portion of **1** were weak, a long range ^{1}H-^{13}C correlation experiment optimized for 10 Hz (XHCORR) (6) was carried out to confirm the aromatic assignments (Figure 1).

The three oxygens required by the molecular formula were positioned on C-2′, C-3′, and C-6′ based upon the chemical shifts of the carbons observed at δ 151.3 (s), 139.7 (s), and 139.4 (s) (7). A long range ^{13}C-^{1}H correlation observed between the methoxyl protons and the carbon observed at 151.3 ppm located the methoxy on C-6′. A long range ^{1}H-^{13}C correlation observed between the exchangeable proton observed at 5.12 ppm and carbons (C-2′, C-3′, and C-4′) assigned a hydroxyl group to C-3′. To satisfy the sites of unsaturation required by the molecular formula and to explain the chemical shift of C-10 (83.8 ppm), **1** must have an ether linkage joining C-2′ and C-10. This completes the structure of **1**.

The relative stereochemistry of **1** was found to be the same as that reported for aureol through a series of difference nOe experiments. The following enhancements, the most illustrative of which are shown in Figure 2, support the assigned stereochemistry. Irradiation of H-14β enhances the resonances observed for H-13abc and H_{eq}-8. Irradiation of H-14α enhances the resonances observed for H-5, H-8, and H_{ax}-7. Irradiation of the Me-15 protons enhances the resonance observed for H-1ab, which suggests that Me-15 is axial. Irradiation of the equatorial methyl protons H-12abc enhances the resonance observed for H-5, while irradiation of the axial methyl protons, H-11abc, enhances the resonances observed for H-5 and H_{ax}-2. Irradiation of the phenolic proton observed at 5.12 ppm enhanced the resonance observed for the H-11 methyl protons.

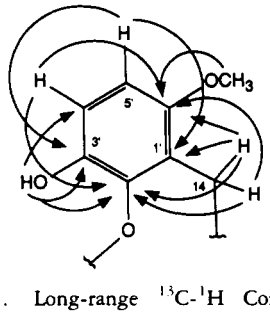


FIGURE 1. Long-range ^{13}C-^{1}H Correlations from XHCORR for the aromatic portion of **1**.

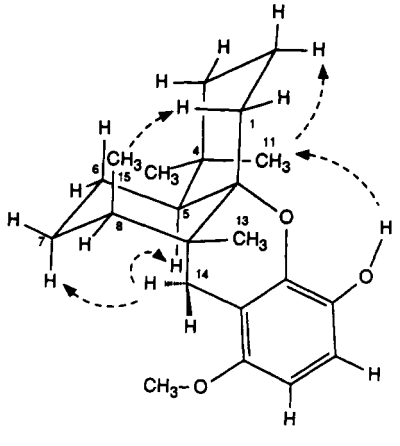


FIGURE 2. Selected nOe enhancements observed for **1**.

Compound **1** is a new member of the growing class of sesquiterpene hydroquinones reported from marine sponges. Similar to puuupehenone, compounds **1** and **2** exhibit cytotoxic activity against a P-388 murine leukemia tumor cell line with IC_{50}'s of 13 and 23.8 μg/ml, re-

spectively (8). Both compounds are active in vitro against influenza virus (strain PR-8) with IC_{50}'s of 6.5 μg/ml (TI = 9) for **1** and 5 μg/ml (TI = 100) for **2**. Full details of the antiviral assays will be published elsewhere.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectral data were measured on the following instruments: ir Perkin-Elmer 1310; uv/visible Perkin-Elmer Lamda 3B; nmr Bruker AM360 with the Aspect 3000 computer; hreims at 70 eV on a Kratos MS-80RFA (Chemical Instrumentation Center, Yale University); [α] Jasco DIP-360 Digital polarimeter. ^{1}H-nmr chemical shifts are reported as δ values in ppm relative to TMS (0 ppm). ^{13}C-nmr chemical shifts are reported as δ values in ppm relative to C_6D_6 (128.0 ppm). ^{13}C multiplicities were measured using the DEPT sequence. INADEQUATE spectra were obtained overnight on approximately 260 mg of material dissolved in 0.5 ml C_6D_6.

COLLECTION OF *S. HARTMANI*.—The sponge, sample number 16-XI-87-1-003, was collected by Johnson Sea Link submersible at a depth of 345 m off Wood Cay, Grand Bahama Island, Bahamas. It was identified as *S. hartmani* by Dr. Shirley Pomponi (1). A voucher specimen has been deposited in the Indian River Coastal Museum located at Harbor Branch Oceanographic Institution, IRCZM catalogue number 003:00037.

ISOLATION OF STRONGYLIN A [**1**].—An extract of 209 g of frozen sponge (stored at −20° until extracted) was prepared as follows: The sponge was placed into a Waring blender with 400 ml of EtOAc and ground for approximately 2 min. The sponge suspension was filtered through Whatman No. 1 filter paper. The sponge residue was returned to the blender for further extraction. This procedure was carried out four times. The combined filtrate was then concentrated by distillation under reduced pressure to yield 2.31 g of a crude brown oil. The crude extract was chromatographed under vlc conditions on a Kieselgel 60H stationary phase using a step gradient of EtOAc/heptane as eluent. Compound **1** was eluted from the column in fractions containing 20 and 30% EtOAc in heptane. These two fractions were combined and rechromatographed under vlc conditions on a Kieselgel 60H stationary phase. A shallow step gradient starting with 100% heptane and proceeding to 40% EtOAc in heptane was used to elute the column. The fraction eluting with EtOAc-heptane (1:9) was substantially pure **1** (>95%). This material could be further purified by hplc on a semi-preparative [1 × 50 cm (1)] Si gel column (Whatman Partisil 10) using 12.5% EtOAc/87.5% heptane as eluent. A flow rate of 4.0 ml/min led to a retention time of 9 min (1.5 column volumes) for **1**. Yield of **1** from 209 g of sponge was 620 mg (0.29% wet wt). Compound **1**: $[\alpha]^{20}D$ +72 (c = 0.023, CH_2Cl_2); ir ν max cm^{-1} (CH_2Cl_2) 3520, 2900, 1600, 1450, 1370, 1312, 1294, 1250, 1170, 1080, 1030, 965, 938, 880, 840; uv nm (MeOH) λ max 276, 215 (ε = 1030, 1370); ^{1}H nmr (C_6H_6-d_6), ca. 150 mg/ml, 360 MHz) δ 6.91 (d, J = 8.6, H-4′), 6.13 (d, J = 8.6, H-5′), 5.12 (s, OH), 3.44 (3H, s, 6′-OMe), 3.20 (d, J = 7.7, H-14α), 2.30 (d, J = 7.7, H-14β), 1.86 (2H, m, H_{ax}-7, H_{ax}-2), 1.66 (2H, m, $H_{ax,eq}$-1), 1.60 (m, H_{eq}-8), 1.43 (m, H-5), 1.41 (m, H_{eq}-6), 1.32 (m, H_{eq}-2), 1.25 (2H, m, H_{ax}-3, H_{ax}-6), 1.12 (2H, m, H_q-7, H_{eq}-3), 1.09 (3H, s, H-11abc), 0.90 (3H, d, J = 7.6, H-15abc), 0.82 (3H, s, H-13abc), 0.65 (3H, s, H-12abc); ^{13}C nmr (C_6D_6, ca. 150 mg/ml, 90 MHz) δ 151.3 (s, C-6′), 139.7 (s, C-2′), 139.4 (s, C-3′), 111.5 (d, C-4′), 110.7 (s, C-1′), 100.8 (d, C-5′), 83.8 (s, C-10), 55.0 (q, 6′-OMe), 44.0 (d, C-5), 39.8 (d, C-8), 38.3 (s, C-9), 34.0 (t, C-3), 33.5 (s, C-4), 32.8 (t, C-14), 32.1 (q, C-12), 29.5 (t, C-1), 29.2 (q, C-11), 27.9 (t, C-7), 22.7 (t, C-6), 20.5 (q, C-13), 18.9 (t, C-2), 17.4 (q, C-15); hrms (70 eV) *m/z* [M]$^+$ 344.2357 (calcd for $C_{22}H_{32}O_4$, 344.2353).

PREPARATION OF **2**.—Compound **1** (15 mg) was placed into a 1-ml Reactivial containing 0.3 ml pyridine (Aldrich) and 0.3 ml Ac_2O (Aldrich). The reaction was allowed to proceed at room temperature for 14 h. After standard workup, compound **2** (14 mg) was obtained. Compound **2**: $[\alpha]^{20}D$ +74 (c = 0.0028, CH_2Cl_2); ir ν max cm^{-1} (CCl_4) 2900, 1745, 1590, 1450, 1360, 1330, 1275, 1180, 1080, 1030, 940, 905, 872; uv nm (MeOH) λ max 272, 215 (ε = 1500, 2725); ^{1}H nmr ($CDCl_3$, ca. 20 mg/ml) δ 6.82 (d, J = 8.7, H-4′), 6.30 (d, J = 8.7, H-5′), 3.80 (3H, s, 6′-OMe), 3.08 (d, J = 17.6, H-14), 2.27 (3H, s, OCO*Me*), 2.14 (d, J = 17.6, H-14), 2.04 (m, H-7), 1.98 (m, H-2), 1.81 (2H, m, H-1ab), 1.76 (m, H-8), 1.68 (m, H-6), 1.48 (2H, m, H-2, H-6), 1.46 (m, H-5), 1.41 (m, H-3), 1.33 (m, H-7), 1.21 (m, H-3), 1.10 (3H, d, J = 7.5 Hz, H-15abc), 1.06 (3H, s, H-11abc), 0.89 (3H, s, H-13abc), 0.80 (3H, s, H-12abc); ^{13}C nmr ($CDCl_3$, ca. 20 mg/ml) δ 168.9 (s, OCOMe), 155.0 (s, C-6′), 143.5 (s, C-2′), 132.6 (s, C-3′), 119.0 (d, C-4′), 111.1 (s, C-1′), 99.5 (d, C-5′), 83.1 (s, C-10), 55.2 (q, 6′-OMe), 44.1 (d, C-5), 39.1 (d, C-8), 37.4 (s, C-9), 33.7 (t, C-3), 33.4 (s, C-4), 31.9 (t, C-14), 31.9 (q, C-12), 29.2 (t, C-1), 28.6 (s, C-11), 27.7 (t, C-7), 22.2 (t, C-6), 20.6 (q, OCO*Me*), 19.9 (q, C-13), 18.3 (t, C-2), 17.2 (q, C-15); hrms (70 eV) *m/z* [M]$^+$ 386.2464 (calcd for $C_{24}H_{34}O_4$, 386.2458).

ANTIVIRAL ASSAY OF **1** AND **2**.—The assay is a cytopathic effect (CPE) reduction assay based upon dye uptake of normal viable Madin-Darby canine kidney cells (MDCK) compared to dye uptake by cells infected with PR-8 virus. MDCK are infected with a viral dose that kills the cell population. Compounds with antiviral activity are identified by a decrease in CPE compared to viral controls. MDCK cell culture was obtained from American Type Culture Collection [ATCC No. CCL 34, MDCK (NBL2-)]. Myxovirus Influenza A strain A/PR/8/34 (HINI) ATCC VR 95 was obtained from American Type Culture collection. Tissue culture plates (96 well) are planted at a cell concentration of 25,000 to 30,000 cells per well and grown for two days. Medium is withdrawn with an eight place manifold and 100 μl of maintenance medium is added to rows B, D, F, and H. Virus (100 μl) diluted in maintenance medium is added to rows A, C, E, and G. After a 1 h incubation period at 37°, 100 μl of the test sample is added. The virus suspension is left on the cell layer. The plates are incubated for three days at 37°. At this time, plates are inspected under the microscope for the overall condition of the MDCK cells and the progression of cytopathic effects in the viral controls. If the plates are in good condition, they are stained with neutral red and the optical densities are measured at 540 nm (9). Maintenance medium is prepared as follows: to Dulbecco's modified EMEM with 1-glutamine and glucose (4.5 g/liter), add additional 1-glutamine, 5 ml of 200 mM/500 ml of medium, 1 ml of 1% nonessential amino acids/100 ml medium, 1 ml of 1% sodium pyruvate/100 ml medium, 50 μg gentamicin/ml medium, and 1 μg trypsin/ml medium.

ACKNOWLEDGMENTS

We thank Dr. Shirley Pomponi (HBOI) for the sponge identification and Dan Pentek (Yale University) for the mass spectral analysis. This is Harbor Branch Oceanographic Institution Contribution Number 849.

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Received 12 October 1990