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Long-Chain Acetylenic Ketones from the Micronesian Sponge Haliclona sp. Importance of the 1-yn-3-ol Group for Antitumor Activity

Guang-Xiong Zhou and Tadeusz F. Molinski *

Department of Chemistry, University of California, Davis, California 95616, USA. Tel. (530) 752-6358, Fax (530) 752-8995

* Author to whom correspondence should be addressed; E-mail: tfmolinski@ucdavis.edu

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Abstract: Two new long-chain C_{33} polyacetylenic compounds, halicynones A and B were isolated from the marine sponge *Haliclona* sp. along with known analogs. The known compound pellynol A possessing a 1-yn-3-ol terminus, exhibited strong antitumor activity against the human colon tumor cell line HCT-116 (IC_{50} 0.026 μg/mL), however, the corresponding 1-yn-3-one, halicynone A, was inactive, which suggests an important role for the terminal 1-yn-3-ol functional group in mediating cytotoxic activity.

Keywords: Porifera, ene-yne, HCT-116, cytotoxic activity, polyacetylene, pellynol.

Introduction

Long-chain polyacetylenes are known from marine sponges of the order Haplosclerida [1]. The molecular structures vary in chain length from C_{33}-C_{46} and are substituted with varying numbers of hydroxyl groups, typically at C_1 and ω-3 (third last carbon from the terminus). The long-chain alkynes found in *Petrosia*, *Pellina*, *Haliclona* and other genera often possess remarkable cytotoxic activity against tumor cells that is not easily explained by the simple functionality that adorns the structures of these

molecules, nor dismissed by non-specific lipid aggregations under assay conditions (e.g. micelles). A recent report that demonstrates that analogs of the polyacetylene petrosynol, from *Petrosia* sp., directly inhibit DNA replication at the level of initiation supports a suggestion that the compounds interfere with proteins required to establish initiation forks [2]. Indeed, earlier studies show that petrosynol inhibits both RNA- and DNA-dependent DNA polymerase [3]. Nevertheless, a unifying structural model to explain these unusually high activities has not been advanced. In our screening for compounds that induce apoptosis (programmed cell-death) in tumor cell lines, an extract of the marine sponge *Haliclona* sp., collected in Micronesia, showed weak antifungal activity against *Candida glabrata* and high cytotoxicity against human colon tumor cells, HCT-116 (1.4% survival at 26 μg/mL). We report here two new acetylenic ketones, **1** and **2**, that are related to the highly cytotoxic long-chain polyacetylenes, pellynols [4] and triangulynes [5] from *Pellina triangulata*. Measurement of structure-activity relationships of **1**, **2** and related compounds **3-9** shows that a terminal 1-yn-3-ol group [6] – but not a 1-yn-3-one – imparts cytotoxic activity to this class of molecules.

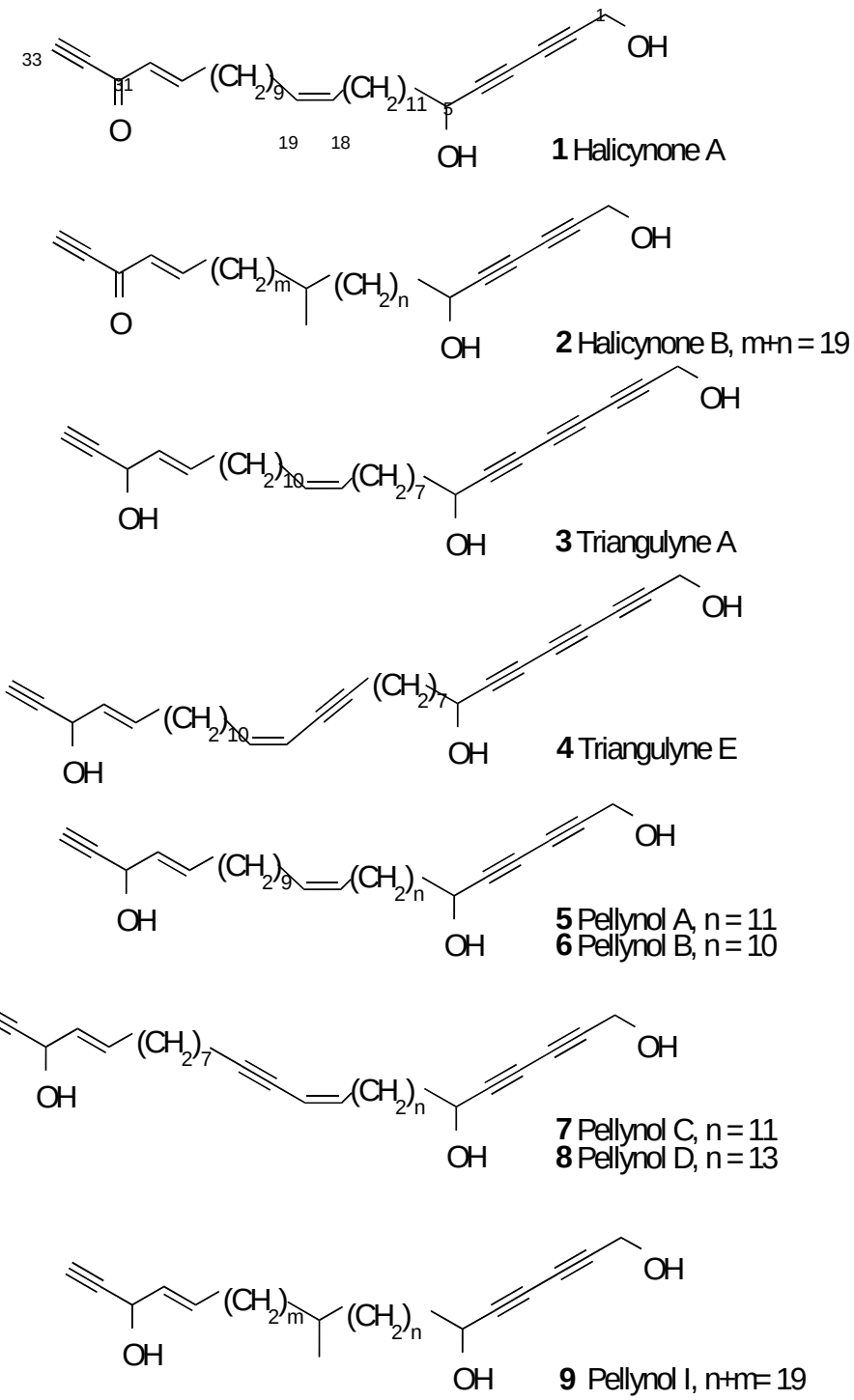
Results and Discussion

Extraction of *Haliclona* sp. followed by chromatographic separation of the solvent-partitioned fractions gave two new compounds halicynones A (**1**) and B (**2**) along with the known compounds triangulyne A (**3**), triangulyne E (**4**) [5] and pellynols [4a,b] A (**5**), B (**6**), C (**7**), D (**8**), and I (**9**) [4c] (0.011-0.11% of dry weight) (Scheme 1). The known compounds were identified by comparison of their NMR and MS data with those of literature values.

The formula of halicynone A (**1**), $C_{33}H_{50}O_3$ (HRFABMS, *m/z* 517.3702, $[M+Na]^+$ Δmmu = 0.4) required nine degrees of unsaturation which were fully accounted for by two carbon-carbon double bonds, three triple bonds and one keto group. Conjugation in the structure of **1** was indicated by UV maxima at λ 228 nm and 254 nm. Examination of the ^{1}H- and ^{13}C-NMR spectral data of **1** revealed the presence of two disubstituted olefinic double bonds (δ_H 5.33, m, 2H, H18,19; δ_C 129.8, 129.0, 2xCH, C18,19; δ_H 7.26, dt, *J*=16,6.8 Hz, 1H, H29; 6.16, dt, *J*=16, 1.2 Hz, 1H, H30; δ_C 156.1, d, C29; 131.9, d, C30), two internal triple bonds (δ_C 78.9, s, C2; 69.8, s, C3; 68.8, s, C4; 80.5, s, C5) and a terminal acetylenic ketone (δ_H 3.20, s, 1H, H33. δ_C 74.0, d, C33; 65.8, s, C32; 178.0, s, C31). The latter was supported by the presence of an IR carbonyl band at ν 1737 cm^{-1}. Two propargylic alcohols accounted for the balance of oxygen in the formula of **1**; a secondary OH at C6 and a primary OH at C1 (δ_H 4.41, t, *J* = 6.8 Hz, H6; δ_C 62.8, d, C6; δ_H 4.33, bs, 2H, H1; δ_C 51.5, t, C1). These assignments were substantiated by the downfield ^{1}H- chemical shifts of these carbinol signals, which are typical for allylic and properyglic alcohols, and HMBC correlations from H1 to C2 and C3 and from H6 to C3, C4 and C7. The cross conjugated ene-yn-one group, C29-C33, in **1** was supported by HMBC correlations from the β-vinyl proton, H29 and the terminal acetylenic proton H33 (δ

3.20, s, 1H) to the keto group, C31. The rest of the NMR signals of **1** were assigned to linear methylene-chain segments that were largely unresolved by NMR.

Scheme 1.



The configuration of the *E*- double bond at C29-30 and *Z*- double bond at C18-19 in **1** followed from observation of a large vicinal coupling constant between the vinyl protons H29 and H30 (J= 14.8 Hz) and the upfield shifts of the allylic CH_2 groups C17 and C20 (δ_C 27.2, t; 27.2, t), respectively. Although location of the *Z*-double bond was not explicitly indicated by the spectroscopic data, it was supported by selective oxidation (MnO_2, CH_2Cl_2) of pellynol A (**5**) to **1**. The product of oxidation was identical to natural **1** by ^{1}H NMR, ESIMS, HPLC retention time and co-injection with an authentic sample (C_{18} HPLC, Dynamax, 4.6x250 mm, 88:12 MeOH/H_2O, rt 32.1 min).

Compound **2**, $C_{32}H_{50}O_3$ was obtained in only small amounts and the structure assigned on the basis of comparisons with pellynol I (**9**). The *Z*- double bond present in **1** was replaced in **2** by signals due to a methyl branch (δ_H 0.83, d, J= 6.8 Hz, 3H); a substitution pattern that is also seen in **9**. All other signals were identical with those of **9**. Although insufficient material was available for ^{13}C-NMR or chemical correlation, the assignments of the functional groups were strongly supported by the presence of identical ^{1}H NMR signals in **1** and **2** for the cross-conjugated acetylenic enone and the terminal propargylic CH_2OH group at C1. Thus, the structure of **2** is highly suggestive of dehydro-pellynol I, however, as with the known parent compound **9** [4c], we were unable to assign the position of the methyl branch from electron impact MS fragmentation data.

The polyacetylenes from *Haliclona* sp. were tested for cytotoxicity. Cultured HCT-116 cells were very sensitive to compounds **5** (IC_{50} 0.026 μg/mL), **6** (0.12 μg/mL), **7** (0.127 μg/mL), **8** (0.103 μg/mL), **9** (<0.008 μg/mL), however, they are unaffected by the acetylenic ketones **1** and **2** (IC_{50} >78 μg/mL) [7, 8]. The unusually high cytotoxicities of **5**-**9** but lack of activity in **1** and **2** suggests a relatively rigid, rod-like molecule is a fundamental requirement for this biological property, but only if the 1-yn-3-ol is present. The importance of the 1-yn-3-ol has been demonstrated in other bioactivity relationships. Recently, long-chain unsaturated 1-yn-3-ols have been reported to induce neurite growth in phenochromocytoma PC12 and neuroblastoma Neuro 2A cells [9,10]. Kobayashi and coworkers reported structure-activity results that show neuritogenic activity is critically dependent upon the presence of the 1-yn-3-ol terminus within a long-chain hydrocarbon, but independent of the presence of internal or ω-terminal ene-yne unsaturation [11].

Pellynols A-D, F and I showed strong cytotoxicity against several melanoma and ovarian cancer cell lines (IC_{50} 0.08-2.0 μM) [4c], but no simple correlation with chain-length or positions of chain oxidation was established. In the present work we reveal that the terminal 1-yn-3-ol functionality is critical in eliciting *in vitro* antitumor activity as oxidation of the terminal propargylic alcohol in **5** to the conjugated acetylenic ketone **1** abolishes activity.

Conclusions

Two new long-chain unsaturated acetylenic ketones, **1** and **2**, were identified from the marine sponge *Haliclona* sp. and correlated with known compounds. Both compounds exhibit unusually high cytotoxicity similar to that reported for other long-chain ene-ynes from Porifera, however, the critical role of the 1-yn-3-ol for cytotoxic activity is now revealed. It is unlikely that long-chain ene-ynes will find utility as antitumor drugs, but the intriguing recurrence of reports of cytotoxicity within this class of compounds merits investigation of their cytological effects.

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Experimental

General

^{1}H- and ^{13}C-NMR spectra were obtained using a Varian Inova 400 NMR spectrometer at 400 and 100 MHz, respectively. Solvents used in extraction or chromatography were HPLC-grade or distilled from glass. ESIMS was carried out on a ThermoFinnigan Surveryor LC and LC Deca ion-trap with infusion in MeOH (0.1% acetic acid). HRMS results were obtained from University of California, Riverside Mass Spectrometry Facility. General experimental procedures are described elsewhere [12]. Antifungal assays were carried out using a modification of a standard microtiter broth dilution assay [13]. Cytotoxicity assays against human colon tumor cells were performed at Scripps Institution of Oceanography (La Jolla, California) using cultured HCT-116 cells incubated with MTS [14]. The endpoint and cell viabilities were determined by measurement of the *soluble* formazan product (λ 490 nm) using a Molecular Devices Spectramax microplate reader [15].

The sponge *Haliclona* sp. (01-09-026) was collected by hand using scuba at a depth of 20m in Pohnpei (07˚ 00'N, 158˚ 17.273'E, Federated States of Micronesia) in September 2001 and kept frozen until needed. The lyophilized tissue (31.8 g) was exhaustively extracted with MeOH and the combined solvent extracts partitioned progressively against hexanes, $CHCl_3$ and *n*-BuOH after adjustment of the H_2O content at each

step. The $CHCl_3$-soluble fraction (140 mg), which exhibited weak antifungal activity, was further separated by gradient silica chromatography (40-63 μm silica, EtOAc in hexanes, then MeOH in EtOAc). Active fractions were separated by HPLC (C_{18} reversed phase, Dynamax 5μ, 300 x 10 mm, 83:17 MeOH/H_2O followed by a gradient of 33:67 to 30:70 H_2O/CH_3CN) to obtain the new compounds halicynone A (**1**, 2.0 mg) and halicynone B (**2**, ~0.5 mg) along with the known compounds triangulyne A (**3**, 10.0 mg, 0.031% of dry weight), triangulyne E (**4**, 6.6 mg, 0.02%) and pellynols A (**5**, 35 mg, 0.11%), B (**6**, 3.7 mg, 0.012%), C (**7**, 4.8 mg, 0.015%), D (**8**, 3.7 mg, 0.011%), and I (**9**, 4.1 mg, 0.012%). The known compounds were identified by comparison of ^{1}H-, ^{13}C-NMR and MS data with the reported literature values [4, 5].

Spectral Data

Halicynone A (**1**), colorless oil; $[\alpha]_D$ +68.6˚ (*c* 0.035, $CHCl_3$); UV (MeOH): λ_{max} 228, 254 nm; IR (film) ν 2296, 2297, 2923, 2852, 2098, 1737, 1648, 1621, cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.26 (1H, dd, *J*=16.0, 6.8 Hz, H-29), 6.16 (1H, dt, *J*=16.0, 1.2 Hz, H-30), 5.33 (2H, t, *J*=6.8 Hz, H-18, 19), 4.41 (1H, t, *J*=6.8 Hz, H-6), 4.33 (2H, brs, H-1), 3.20 (1H, s, H-33), 2.29 (2H, dq, *J*=6.8, 1.6 Hz, H-28), 2.00 (4H, q, *J*=7.6 Hz, H-17, 20), 1.68 (2H, m, H-7), 1.40 (2H, m, H-8), 1.22 (brs, CH_2), 1.50 (2H, m, H-27); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm) 178.0 (s, C-31), 131.9 (d, C-30), 129.8 (d, C-18), 129.9 (d, C-19), 80.5 (C-5), 78.9 (s, C-2), 74.0 (d, C-33), 69.8 (s, C-3), 68.8 (s, C-4) 65.8 (s, C-32), 62.8 (d, C-6), 56.1 (d, C-29), 51.5 (C-1), 37.5 (t, C-7), 32.7 (t, C-28), 25.0 (t, C-8), 29.7-29.2 (t, C-9-16, 21-26), 27.2 (2xt, C-17, C-20); 27.7 (t, C-27); ESIMS *m/z* 517.7 ($[M+Na]^+$); HRFABMS: *m/z* 517.3702 ($[M+Na]^+$), calcd. 517.3658 for $C_{33}H_{50}O_3Na$.

Halicynone B (**2**), colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.26 (1H, *J*=15.6 Hz, H-27), 6.16 (1H, dt, *J*=15.6, 6.8 Hz, H-28), 4.41 (1H, t, *J*=6.6 Hz, H-6), 4.33 (2H, s, H-1), 3.20 (1H, s, H-31), 2.30 (1H, dq, *J*=6.8, 1.6 Hz, H-26), 1.68 (2H, m, H-7), 1.50 (2H, m, H-25), 1.22 (m, CH_2), 0.83 (3H, d, *J*=6.8 Hz); ESIMS *m/z* 505.6 ($[M+Na]^+$); HRFABMS: *m/z* 505.3653 ($[M+Na]^+$); Calcd. 505.3657 for $C_{32}H_{50}O_3Na$.

*Oxidation of Pellynol A (**5**) with MnO_2: Conversion to 1*

A solution of pellynol A (**5**, 4.0 mg) in CH_2Cl_2 (1.0 mL) was stirred at room temperature with MnO_2 (10 mg) and monitored by TLC (1:20 MeOH/CH_2Cl_2) until the starting material disappeared. The mixture was filtered, concentrated and applied to a short column of silica (400 mg) and eluted with 1:7 EtOAc/hexane provided compound **1** (1.5 mg, 38%). The product was shown to be identical with authentic natural product by ^{1}H-NMR, ESIMS and HPLC (C_{18} reversed phase, Dynamax, 85:15 MeOH/H_2O, rt 32.1 min).

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6. Nomenclature considerations define the primary alcohol in pellynol A (**5**) at C1 and the terminal acetylene carbon at C33. Systematic numbering of the corresponding ketones **1** and **2** would require reversed ordering of locant numbers. In order to emphasis the importance of the terminal propargylic alcohol and retain a useful reference point, we use the terms '1-yn-3-ol' and '1-yn-3-one' to refer to functionality numbered from the ω-terminus with respect to pellynol numbering.
7. Compounds **2**, **3**, and **4** were not tested against HCT-116 due to insufficient material or decomposition.
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15. Compounds were assayed with compounds in DMSO (final concentration, 1% v/v) and run against etoposide as positive control. HCT-116 cells were incubated in 96-well plates for 72 h before addition of MTS. Well absorbances (λ 490 nm) were corrected for background and expressed as a percentage of the negative control (DMSO, only).

Sample Availability: Samples are available from the authors.