

13-Epi-9-deacetoxyxenicin, a Cytotoxic Diterpene from the Soft Coral *Asterospicularia laurae* (Alcyonacea)

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Abstract: An investigation of the soft coral *Asterospicularia laurae* collected on the Great Barrier Reef, Australia, afforded the cytotoxic diterpene 13-*epi*-9-deacetoxyxenicin (**1**) in addition to the known metabolites 13-*epi*-9-deacetylxenicin (**2**) and gorgosterol. 13-*Epi*-9-deacetoxyxenicin readily underwent an autoxidation reaction in solution to afford a single product, the hydroperoxide **3**. Structures, stereochemistry, and NMR assignments were established by high resolution NMR spectroscopy and by comparison with published data for known compounds.

Keywords: Cytotoxic, xenicane diterpene, soft coral, *Asterospicularia*.

Introduction

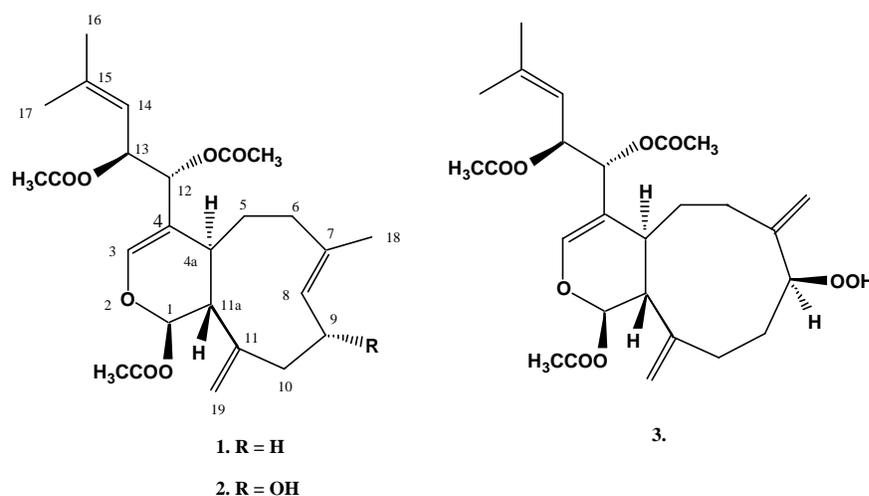
Asterospicularia laurae Utinomi is a moderately abundant soft coral species found in well lit areas of the Great Barrier Reef, especially on reef flats. Fabricius and Alderslade [1] have remarked upon the similarity between the polyp structure of *Asterospicularia* and those of the genera *Xenia* and *Symphodium*. The chemistry of only one member of the genus *Asterospicularia* has been reported to date: Ksebati and

Schmitz [2] reported in 1984 the isolation of 24-methyl-5 α -cholestane-3 β , 5, 6 β , 22R, 24-pentol 6-acetate from *Asterospicularia randalli*. Here we report the isolation of diterpenes related to xenicin from *A. laurae*.

Xenicin was the first reported marine metabolite with the xenicane diterpenoid skeleton [3], a skeleton characterised by a 9-membered carbocyclic ring fused to a 6-membered oxygen-containing ring that is formally derived from cyclization of two aldehyde functions. Since the initial report, a large number of related metabolites have been isolated, with the 6-membered ring usually either formed from an enol-acetal or lactone linkage. The majority of these metabolites have been isolated from the soft coral genus *Xenia* [4-11], but others have been reported from the soft coral genera *Anthelia* [12, 13], *Alcyonium* [13], and *Capnella* [14], from the blue coral *Heliopora coerulea* [15], and from gorgonians [16].

Results and Discussion

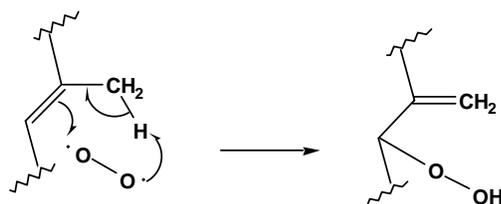
Chromatography of the dichloromethane extract of *Asterospicularia laurae* afforded gorgosterol, the new diterpenoid 13-*epi*-9-deacetoxyxenicin (**1**), and the known xenicane diterpenoid 13-*epi*-9-deacetylxenicin (**2**) [5a].



The formula of **1** was confirmed as C₂₆H₃₆O₇ by high resolution mass measurement of the [M+Na]⁺ ion and the structure and stereochemistry were determined by comparison of its spectral data with those obtained for 13-*epi*-9-deacetylxenicin. The principal difference in the ¹H-NMR spectra was the absence for **1** of the allylic oxymethine signal that resonated at δ 4.72 for (**2**). ¹H- and ¹³C-NMR assignments (Table 1) were determined from gCOSY, gHMBC, and gHMBC NMR spectral data.

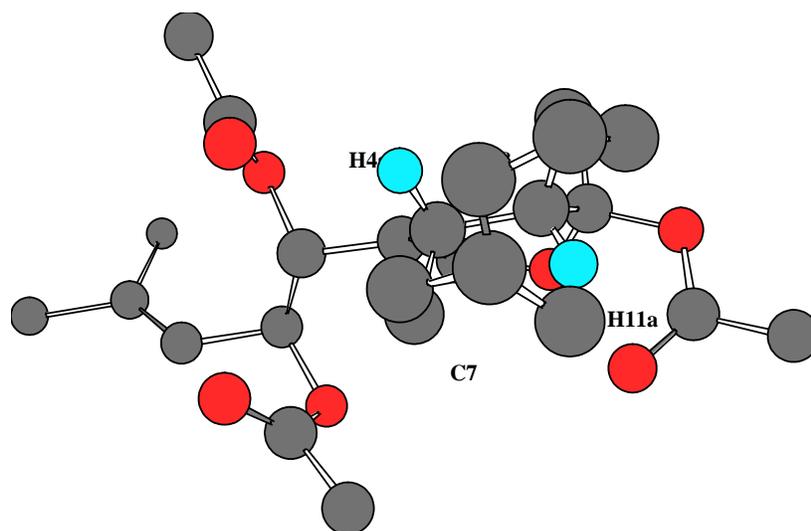
The stability of **1** in solution differed significantly from that of 13-*epi*-9-deacetylxenicin (**2**), as it readily underwent autoxidation (an Ene reaction, see Figure 1) to afford a single hydroperoxide product, while solutions of 13-*epi*-9-deacetylxenicin (**2**) were relatively stable.

Figure 1. Autoxidation of an alkene - an Ene reaction



The stereochemistry of the 8-hydroperoxy group in **3** is consistent with oxygen addition from the least hindered face of the alkene. It should be noted that single crystal x-ray structures of xenicane diterpenes like xenicin [3], indicate that the $\Delta^{7,8}$ bond lies orthogonal to the ring junction. This would be expected to prevent approach for endo addition to the olefinic bond. Indeed, the coupling constants (5.0 and 10.4 Hz) observed for H8 in **3** are consistent with exo addition, and a conformation similar to that determined for 13-*epi*-9-deacetylxenicin by single crystal x-ray crystallography [5a]. Further evidence for a conformation of 13-*epi*-9-deacetylxenicin with the $\Delta^{7,8}$ bond orthogonal to the ring junction (see Figure 2) is provided by the shielding of the 4a and 11a $^1\text{H-NMR}$ signals relative to those observed for the hydroperoxide (**3**).

Figure 2. Computer generated proposed conformation of 13-*epi*-9-deacetylxenicin showing the orthogonal arrangement of the C7-C8 bond relative to the C4a-C11a ring junction.



Cytotoxicity assays were carried out against a cultured suspension of P388D₁ mouse lymphoma cells. 13-*Epi*-9-deacetoxyxenicin (**1**) exhibited an IC₅₀ of 0.1 µg/ml, while the IC₅₀ of 13-*epi*-9-deacetylxenicin (**2**) was approximately 1.0 µg/ml.

Conclusions

The soft coral *Asterospicularia laurae* from Old and Myrmidon Reefs, Great Barrier Reef, Australia contains the cytotoxic diterpenes 13-*epi*-9-deacetylxenicin and 13-*epi*-9-deacetoxyxenicin. 13-*Epi*-9-deacetoxyxenicin readily undergoes an autoxidation reaction to form a hydroperoxide.

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Experimental

General

Melting points were determined in a Stuart SMP1 apparatus and are uncorrected. Optical rotations were measured in CHCl₃ with a PolAAr 2001 polarimeter. Mass spectral data were determined on a Bruker BioAPEX 47e mass spectrometer operating in positive ion electrospray mode at the Australian Institute of Marine Science, Cape Ferguson. ¹H-NMR spectra were measured in CDCl₃ at 300 MHz and ¹³C-NMR spectra at 75.5 MHz on a Varian Mercury NMR using residual solvent peaks for calibration. gCOSY, gHMQC and gHMBC spectra were obtained using the standard Varian pulse sequences optimised for ¹J= 140Hz, and ^{2,3}J= 8Hz. Merck t.l.c. grade silica gel (type 60) was used for column chromatography. All solvents used were freshly distilled.

Animal material

Samples of the soft coral *Asterospicularia laurae* were collected by hand using scuba (at a depth of 2-5m) at Old and Myrmidon Reefs (Great Barrier Reef, Australia), frozen immediately after collection and kept frozen until used. A voucher specimen (NTM C12570) has been deposited in the Museum and Art Gallery of the Northern Territory, Darwin.

Extraction and Purification

The frozen coral was freeze dried and the dried material (206.1 g) was extracted with dichloromethane (3 X 1.5 L). Solvent removal afforded the dichloromethane extract (7.4 g, 3.6%). The residue was then twice extracted with methanol. The methanol extract was evaporated and partitioned between water and dichloromethane. Removal of the dichloromethane afforded the methanol lipophilic extract (1.6 g, 0.8%), which was combined with the dichloromethane extract. A portion (3.7 g) of the combined extract was repeatedly subjected to vacuum liquid chromatography on silica gel [17] eluted with dichloromethane-ethyl acetate, hexane-ethyl acetate and hexane-diethyl ether mixtures for successive columns to afford gorgosterol (39 mg, 0.038%), 13-*epi*-9-deacetoxyxenicin (**1**) (44 mg, 0.043%) and 13-*epi*-9-deacetylxenicin (**2**) (106mg, 0.103%). 13-*Epi*-9-deacetoxyxenicin (**1**) crystallised from cold hexane in prisms m.p. 86-87°, $[\alpha]_D +63^\circ$ (c. 0.06, CHCl₃). 13-*Epi*-9-deacetylxenicin (**2**) crystallised from diethyl ether in prisms m.p. 150-151°, $[\alpha]_D +51^\circ$ (c. 0.11, CHCl₃) {lit. m.p. 142-4°, lit $[\alpha]_D +44^\circ$ (c. 0.48, CH₂Cl₂) [5a]}. Solutions of **1** in dichloromethane or ether underwent autoxidation to afford a single product that was identified as **3** and obtained as an oil $[\alpha]_D +48^\circ$ (c. 0.09, CHCl₃).

Spectral Data

13-*Epi*-9-deacetoxyxenicin (1): ¹H- and ¹³C-NMR (CDCl₃): see Table 1. Observed HMBC correlations: 1-Acetate CH₃ to 1-acetate CO; H3 to C1, C4, C4a, C12; H8 to C6, C7, C9, C10, C18; H12 to C3, C4, C4a, C13, C14, 12-OCO; 12-Acetate CH₃ to 12-acetate CO; H13 to 13-OCO, C14; 13-Acetate CH₃ to 13-acetate CO; H14 to C12, C13, C15, C16, C17; H16 to C14, C15, C17; H17 to C14, C15, C16; H18 to C6, C7, C8; H19a/H19b to C10, C11, C11a; IR (Nujol) cm⁻¹: 1761, 1732, 1457, 1373, 1229, 1157, 1027, 959, 928; HRMS (Electrospray) for C₂₆H₃₆O₇Na [MNa]⁺: Calcd 483.2353; Found 483.2341.

13-*Epi*-9-deacetylxenicin (2): ¹H- and ¹³C-NMR spectral data (see Table 1) was in agreement with the published data [5a].

Hydroperoxide (3): ¹H- and ¹³C-NMR: see Table 1. Observed HMBC correlations: 1-Acetate CH₃ to 1-acetate CO; H3 to C1, C4, C4a, C12; H12 to C3, C4, C4a, C13, C14, 12-OCO; 12-Acetate CH₃ to 12-acetate CO; H13 to 13-OCO, C14; 13-Acetate CH₃ to 13-acetate CO; H14 to C16, C17; H16 to C14, C15, C17; H17 to C14, C15, C16; H18a/H18b to C6, C8; H19a/H19b to C10, C11a; HRMS (Electrospray) for C₂₆H₃₆O₉Na [MNa]⁺: Calcd 515.2251; Found 515.2235.

Table 1: NMR Assignments for **1-3** in CDCl₃

Compound	1		2		3	
	Carbon	$\delta^1\text{H}$, mult., <i>J</i> (Hz)	$\delta^{13}\text{C}$	$\delta^1\text{H}$, mult., <i>J</i> (Hz)	$\delta^{13}\text{C}$	$\delta^1\text{H}$, mult., <i>J</i> (Hz)
1	5.87, d, 1.5	91.4	5.86, d, 1.8	91.7	6.08, d, 2.4	92.4
3	6.49, d, 1.8	141.7	6.49, d, 1.5	141.9	6.45, brs	142.0
4		113.6		113.5		111.5
4a	2.2, m	37.1	2.33, brd, 13.8	36.6	2.44, m	32.1
5	1.55, m; 1.95, m	30.3	2.05, m; 1.97, m	30.5	1.78, m; 2.32, m	30.5
6	2.0 - 2.2, m, 2H	39.6	2.2, m	39.5	2.22, m; 2.38, m	25.6
7		135.5		133.0		145.5
8	5.37, brt, 7.9	124.4	5.24, brd, 9.3	131.0	4.41, dd, 5.0, 10.4	90.0
9	2.25, m; 2.45, m	24.9	4.72, brdd, 7.2, 6.0	67.7	2.03, m; 1.64, m	26.5
10	2.15 - 2.25, m	35.4	2.3, m; 2.6, m	46.3	2.12-2.24, m	30.7
11		151.3		147.2		146.6
11a	1.95, m	48.9	1.85, brs	49.4	2.70, brt, 1.5	39.3
12	5.32, d, 6.6	74.9	5.31, d, 6.8	74.9	5.29, d, 7.5	74.8
13	5.68, dd, 6.6, 9.3	70.0	5.69, dd, 6.8, 9.3	69.9	5.59, dd, 7.5, 9.3	69.6
14	5.14, dsept, 9.3, 1.2	119.5	5.13, brd, 9.3	119.5	5.10, brd, 9.3	119.6
15		140.0		140.1		140.1
16	1.74, d, 1.2	25.8	1.74, brs	25.8	1.73, brs	25.9
17	1.75, d, 1.2	18.5	1.74, brs	18.6	1.75, brs	18.6
18	1.65, d, 0.8	16.7	1.64, brs	17.7	5.34, brs; 5.41, brs	119.0
19	4.78, d, 1.2; 4.86, brs	112.9	4.56, brs; 4.84, brs	115.1	4.79, brs, 4.87, brs	112.1
1-OAc	2.03, s	20.9	2.02, s	20.9	2.05, s	21.0
		169.5		169.5		169.4
12-OAc	*2.02, s	21.1	2.01, s	21.1	2.01, s	21.1
		169.8		169.8		169.8
13-OAc	*2.00, s	21.1	2.00, s	21.2	1.95, s	21.0
		169.8		170.0		169.9
8-OOH					8.17, brs	

* 12- and 13-acetate assignments for **1** may be interchanged.

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Sample Availability: Samples are available from the authors.

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