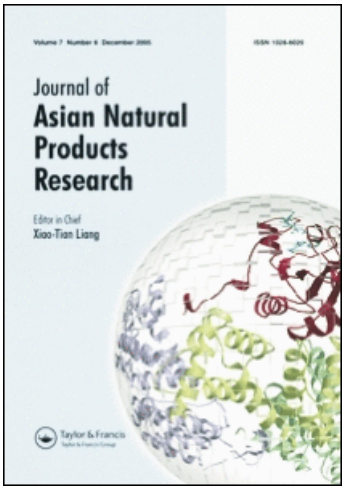


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# Note

# 3-Oxo-axisonitrile-3, a new sesquiterpene isocyanide from the Chinese marine sponge *Acanthella* sp.

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A new sesquiterpene isocyanide, 3-oxo-axisonitrile-3 (**1**), with a spiro [5,6] decane skeleton (spiroaxane) together with a known related sesquiterpene isonitrile (**2**), sesquiterpene isothiocyanates (**3**–**8**) and two diterpene isonitriles (**9**, **10**) have been isolated from the Chinese marine sponge *Acanthella* sp. The structure of **1** has been determined on the basis of spectroscopic analysis.

*Keywords*: Marine sponge; *Acanthella* sp.; Sesquiterpene isocyanide; 3-Oxo-axisonitrile-3

## 1. Introduction

Marine sponges in the family Axinellidae belonging to the genera *Axinella* and *Acanthella* have yielded a wide variety of interesting terpenoid metabolites containing isonitrile, isothiocyanate and formamide [1]. As part of an ongoing investigation of the chemistry of South China Sea marine invertebrates [2,3] we have examined extracts of an undescribed sponge species belonging to the genus *Acanthella* collected off Ximao Island, Hainan Province, China. The extract was found to contain a new sesquiterpenoid, 3-oxo-axisonitrile-3 (**1**), seven known sesquiterpenoids (**2**–**8**), and two known diterpenoids (**9** and **10**) (figure 1). Each of them contained either an isonitrile group or an isothiocyanate moiety. In this paper we describe the isolation and structure elucidation of the new sesquiterpene.

## 2. Results and discussion

The $Et_2O$-soluble fractionation of the acetone extract of the sponge was successively separated by silica-gel flash column chromatography and reversed-phase HPLC to give pure

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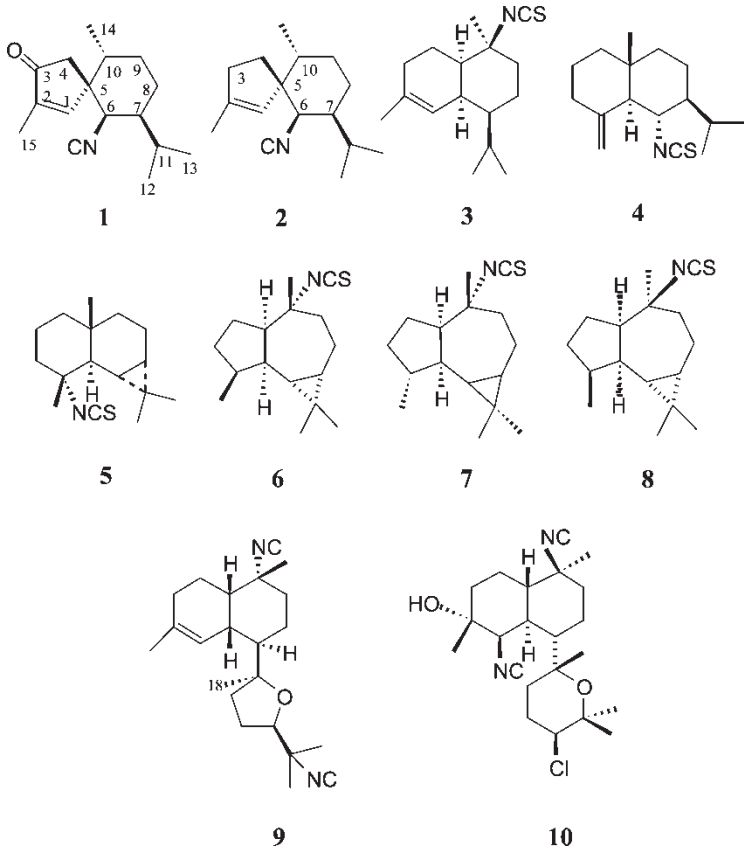


Figure 1. Structure of compounds **1**–**10**.

compounds 3-oxo-axisonitrile-3 (**1**) (1.5 mg from 120 αg dry weight of sponge), axisonitrile-3 (**2**) [4], (1*R*,6*S*,7*S*,10*S*)-10-isothiocyanato-4-amorphene (**3**) [5], acanthine B (**4**) [6], isothiocyanate 1 (**5**) [7], (+)-10*R*-isothiocyanatoalloaromadendrane (**6**) [8], 10α-isothiocyanatoalloaromadendrane (**7**) [9], (+)-axiothiocyanate (**8**) [10], kalihinene (**9**) [11] and kalihiol A (**10**) [12]. The known compounds were identified by comparison of their spectroscopic data with corresponding literature values.

3-Oxo-axisonitrile-3 (**1**), a colourless oil, $[\alpha]_D + 74.0$ (*c* 0.13, $CHCl_3$), has a molecular formula of $C_{16}H_{23}NO$, determined by high-resolution EI mass spectroscopy {*m*/*z* 245.1773 $[M]^+$}. The $^1H$ and $^{13}C$ NMR spectra revealed the presence of four methyls, three methylenes, four methines, one quaternary $sp^3$ carbon ($\delta$ 49.9), one trisubstituted double bond ($\delta$ 145.0, s; $\delta$ 155.7, d), a carbonyl group ($\delta$ 206.4) and an isocyanide ($\delta$ 157.7 brs). The IR absorption at $\nu_{max}$ 2135 $cm^{-1}$ further supports the presence of the isocyanide. The formula of **1** implies two further degrees of unsaturation, in addition to the two accounted for the ketone and the double bond, and two accounted for the $-N^+ \equiv C^-$ group. Therefore, **1** is an oxygenated sesquiterpene isocyanide with two rings. In the $^1H$ NMR spectrum, an olefinic proton ($\delta$ 7.13) and a pair of AB type protons resonating at $\delta$ 2.48 and 2.52 ($J = 1.92$ Hz) suggest that **1** contains a five-membered α,β-unsaturated ketone ring (figure 2), which was evidenced by the IR ($\nu_{max}$ 1704.8 $cm^{-1}$) and UV ($\lambda_{max}$ 228 nm,

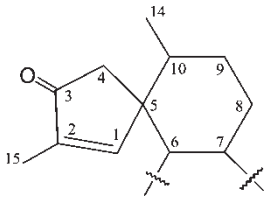


Figure 2. Partial structure A for **1**.

$\varepsilon = 26490$) spectral data. Careful interpretation of the $^1H-^1H$ COSY spectrum with concomitant analysis of the $^1H-^{13}C$ COSY spectrum gave rise to partial structure A for **1**.

Connectivities of C6/C7/C8/C9 and C10/C14 were easily deduced by tracing COSY cross-peaks. Long-range coupling between $H_3$-15 and H-1 places the methyl group at C-2 while $^3J_{CH}$ and $^2J_{CH}$ correlations of H-1/C-2, H-1/C-5, H-4/C-3, H-4/C-5, H-6/C-5, H-1/C-3 and H-1/C-15 in HMBC experiments secure the assignment of all quaternary carbons (figure 3 and table 1).

The remaining portion has a composition of $C_4H_7N$, consisting of an isocyanide and an isopropyl moiety, deduced from the $^1H$ NMR data, in which the methine resonance at $\delta$ 1.60 (H-11) is coupled to two methyl doublets at $\delta$0.92 (d, $J = 6.6$ Hz, Me-12) and 0.99 (d, $J = 6.6$ Hz, Me-13). $^1H-^1H$ COSY correlation also shows that H-11 is coupled to the methine proton resonance at $\delta$1.32 (H-7), which was in turn is correlated with a methine proton resonance at $\delta$ 3.65 (H-6). The downfield chemical shift of H-6 ($\delta$ 3.65) indicates that the isonitrile function is linked to C-6.

Compound **1** showed structural similarity with the co-occurring spiro sesquiterpene **2** (axisonitrile-3) [4]. Careful comparison of the NMR data of **1** with those of **2** (table 1) confirms the proposed structure. The relative configuration of the chiral centres on the six-membered ring of **1** was determined to be same as those of **2** by a combination of NOESY experiments of **1** and analyses of proton coupling constants (figure 4). The NOE effect between H-1 and H-7 not only indicates the stereochemistry at the spiro center, but also reveals the equatorial configuration of the isopropyl group. The splitting pattern of H-6 (brs) and NOE cross-peak of H-6/H-7 indicates an axial orientation for the isocyanide, while the NOE cross-peaks of H-10/H-4, H-10/H-$8_{ax}$ indicate an equatorial position for the methyl at C-10. The absolute stereochemistry of **1** is yet to be determined.

Sesquiterpenes with spiroaxane skeleton are quite rare in nature. The crude extract exhibited cytotoxic activity against murine leukemia P388 cells, but 3-oxo-axisonitrile-3 (**1**)

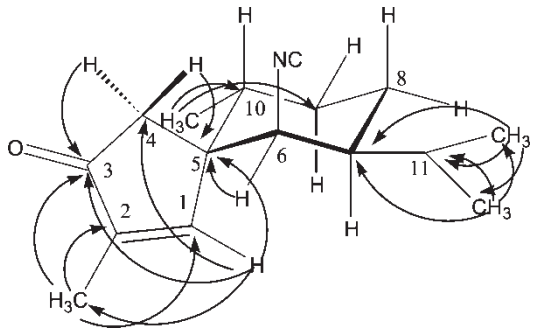


Figure 3. Key HMBC correlations observed for **1**.

Table 1. $^{1}H$ and $^{13}C$ NMR data for **1** and $^{13}C$ NMR data for **2** (in $CHCl_3$)[a].

| | *1* | | |
|---|---|---|---|
| *No.* | *$\delta^{1}H$ (mult. J in Hz)* | *$\delta^{13}C$ (mult.)* | *$\delta^{13}C$ (mult.)* |
| 1 | 7.13 (brs) | 155.7 (d) | 123.5 (d) |
| 2 | – | 145.0 (s) | 144.6 (s) |
| 3 | – | 206.4 (s) | 35.7 (d) |
| 4a | 2.48 (d, 19.2) | 46.6 (t) | 35.1 (t) |
| 4b | 2.52 (d, 19.2) | | |
| 5 | – | 49.9 (s) | 57.0 (s) |
| 6 | 3.65 (brs) | 63.0 (d) | 64.3 (d) |
| 7 | 1.32 (m) | 43.3 (d) | 43.9 (d) |
| $8_{ax}$ | 1.45 (dddd, 4.0, 13.3, 13.3, 13.3) | 24.5 (t) | 24.8 (d) |
| $8_{eq}$ | 1.93 (br dd, 13.3, 4.0) | | |
| $9_{ax}$ | 1.25 (dddd, 4.0, 13.3, 13.3, 13.3) | 30.6 (t) | 31.1 (t) |
| $9_{eq}$ | 1.71 (br dd, 13.3, 4.0) | | |
| 10 | 2.07 (m) | 32.9 (d) | 34.2 (d) |
| 11 | 1.60 (m) | 29.6 (d) | 29.6 (d) |
| 12 | 0.92 (d, 6.6) | 20.2 (q) | 20.2 (q) |
| 13 | 0.99 (d, 6.6) | 20.6 (q) | 20.8 (q) |
| 14 | 0.70 (d, 6.5) | 15.7 (q) | 17.4 (q) |
| 15 | 1.82 (brs) | 10.3 (q) | 16.0 (q) |
| 16 | – | 157.7 (s) | 155.6 (s) |

[a] Bruker AV500; $\delta$ (ppm) referenced to TMS as internal standard. Assignments deduced from analysis of 1D and 2D spectra and comparison with known compound **2**.



proved to be inactive. Compounds **1**–**10** were also tested for inhibitory activity to COX-2 – only kalihiol A (**10**) showed significant activity, with an $IC_{50}$ of 1.07 μM.

## 3. Experimental

### *3.1 General experimental procedures*

UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer; IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer; $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV500 (500 MHz for $^{1}H$ and 125 MHz for $^{13}C$) spectrometer. Chemical shifts ($\delta$) are reported in ppm relative to an internal TMS standard, coupling constants ($J$) are in Hz. $^{1}H$ and $^{13}C$ NMR data were assigned by $^{1}H$–$^{1}H$ COSY, HMQC, HMBC and NOESY experiments. The HR-EIMS spectrum was recorded on a MAT-711 mass spectrometer. Commercial silica gel plates (Qing Dao Hai Yang Chemical Group Co.) were used for TLC.

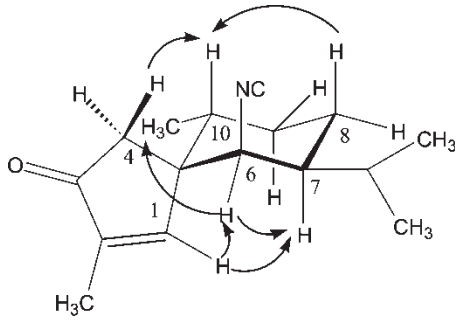


Figure 4. Key NOEs observed for **1**.

Chromatograms were detected by UV lamp at 254 nm, and successively sprayed with 0.1% $Ce(SO_4)_2$ in $2N{\cdot}H_2SO_4$ and heated at 80°C for 5 min.

### *3.2 Collection of biological material*

The examined sample was collected from Ximao Sea, Hainan Province, China in December 2001 and identified by Professor Jin-he Li of the Institute of Oceanology, CAS. Freshly collected sponge tissue was frozen on site and stored at −20°C until workup. A voucher specimen is available for inspection at the Herbarium of Institute of Materia Medica, SIBS-CAS.

### *3.3 Extraction and isolation*

The frozen marine sponge (dry weight 120 g) was extracted with acetone at room temperature. The acetone extract was then concentrated *in vacuo* and the resulting residue partitioned between $H_2O$ and $Et_2O$. The $Et_2O$ extract (3.0 g) was chromatographed on a silica gel column using eluents of increasing polarity from light petroleum to $Et_2O$, MeOH. The fraction eluted with 2% $Et_2O$–light petroleum was further purified by RP-HPLC with MeOH–$H_2O$ (90:10) as eluent, affording **5**–**8**. The fraction eluted with 5% $Et_2O$–light petroleum was further purified by RP-HPLC with MeCN–$H_2O$ (85:15) as eluent, affording **3** and **4**. Compound **2** was obtained as a prism crystal from the fraction eluted with 10% $Et_2O$–light petroleum, the mother solution was further purified by RP-HPLC with MeOH–$H_2O$ (75:25) (3 ml $min^{-1}$, retention time 13.5 min) as eluent to afford **1**. The fractions eluted with 20% and 25% $Et_2O$–light petroleum were further purified by LH-20 (light petroleum–$CHCl_3$–MeOH 2:1:1) as eluent to furnish **9** and **10**, respectively.

**3.3.1 3-oxo-axisonitrile-3 (1)**. A colourless oil, $[\alpha]_D^{20}$ (*c* 0.13, $CHCl_3$); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3388, 2924, 2137, 1705, 1448, 1383, 910; EIMS, *m/z*: 245 ($M^+$), 230, 202, 174; HR-EIMS *m/z* 245.1773 (calcd for $C_{16}H_{23}NO$, 245.1780); UV (MeOH) $\lambda_{max}$ (nm): 228 ($\varepsilon = 26490$); $^1H$ NMR ($CHCl_3$, 500 MHz) see table 1; $^{13}C$ NMR ($CHCl_3$, 125 MHz) see table 1.

## Acknowledgements

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