Haliclocyclin C, a New Monomeric 3-Alkyl Pyridinium Alkaloid from the Arctic Marine Sponge *Haliclona viscosa*

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For the first time, the macrocyclic monomeric 3-alkyl pyridinium alkaloid with an alkyl chain of 13 methylene groups, haliclocyclin C (**1**), could be identified from a natural source in the crude extract of the Arctic sponge *Haliclona viscosa*. Structure elucidation was carried out by comparison of the natural product with the corresponding synthetic compound.

Key words: 3-Alkyl Pyridinium Alkaloid, Cyclic Monomer, HPLC-MS, High-resolution MS

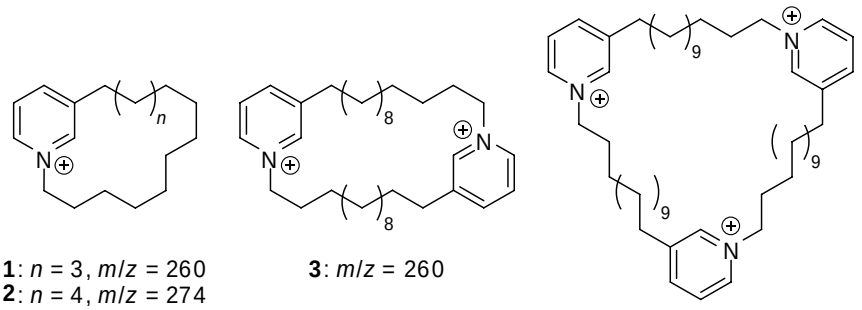
Introduction

In recent years, a number of novel 3-alkyl pyridinium alkaloids were identified from the crude extract of the Arctic marine sponge *Haliclona viscosa* [1 – 5]. The material available from this sponge was limited by the small size of the individuals, their slow growth rate and the effort to sample substainably in the Arctic ecosystem. A new refinement of the spectroscopic methods led to improved resolution of the compound peaks in HPLC experiments and consequently also in the coupled mass spectrometric analyses. As a result, we were able to identify a new monomeric 3-alkyl pyridinium alkaloid (**1**, Scheme 1) by HPLC-HRMS experiments.

Results and Discussion

Compound **1** elutes at a retention time of 15.5 min in the crude extract of *H. viscosa*. It shows a singly charged molecular ion of *m/z* = 260.2368 which suggests a molecular formula of $C_{18}H_{30}N$ (Δm = 1.8 ppm, Fig. 1). Apart from a partly overlapping, unidentified compound, the mass spectrum of the natural compound does not show other molecular, fragment or adduct ions. The molecular mass is by 14 mass units smaller than the mass of the known cyclic monomeric 3-alkyl pyridinium compound **2** which elutes at 17.9 min in the same sponge extract and also shows only a singly charged molecular ion [5]. The crude extract of *H. viscosa* contains a number of compounds that are structurally very similar but differ in the length of the alkyl chains and the degree of oligomerisation. Cyclostellettamine C (**3**) at a retention time of 16.5 min also shows a molecular ion at *m/z* = 260, but it can be differentiated from the molecular ion of **1** by being doubly charged. Another molecular ion of *m/z* = 260 is found for viscosamine C (**4**) [6] which elutes at 17.1 min, but the molecular ion of **4** is triply charged, and the spectrum shows additional fragment and adduct masses [1].

Therefore, it seems reasonable to propose that **1** is the structural analogue of **2** and contains an alkyl chain that is shortened by one methylene group in compar-



1: *n* = 3, *m/z* = 260
2: *n* = 4, *m/z* = 274

3: *m/z* = 260

4: *m/z* = 260

Scheme 1. Cyclic monomeric, dimeric and trimeric 3-alkyl pyridinium alkaloids isolated from the Arctic sponge *Haliclona viscosa*.

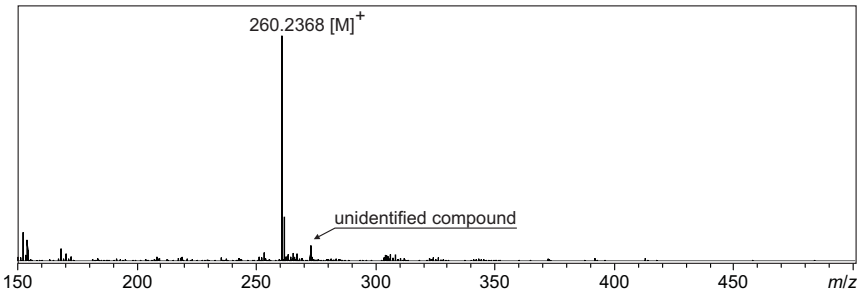


Fig. 1. High-resolution mass spectrum of natural haliclocyclin C (**1**) resulting from a chromatographic separation of the crude extract.

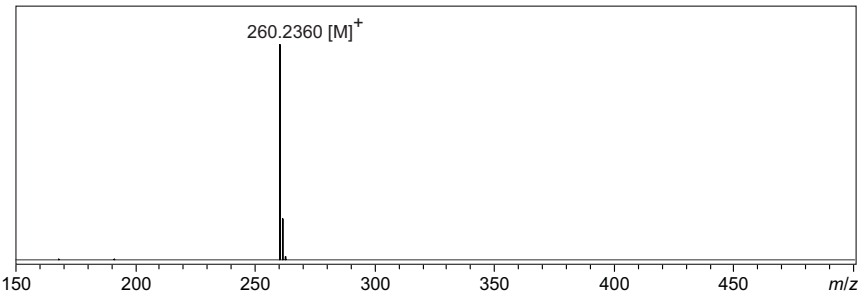


Fig. 2. High-resolution mass spectrum (direct injection) of synthetic haliclocyclin C (**1**).

ison to **2**. Compound **1** has previously been synthesised during the development of the total synthesis of cyclostellettamine C (**3**) [7] as well as for cytotoxicity studies [5] and is therefore available for chromatographic comparisons between the natural and the synthetic compound.

The synthetic compound **1** elutes at the same retention time as the natural compound **1** in the crude extract. The HPLC-HRMS analysis of the synthetic compound **1** yields the mass *m/z* = 260.2360 (Fig. 2) which corresponds to the molecular formula $C_{18}H_{30}N$ with a mass accuracy of 4.9 ppm and fits very well to the observed mass of the natural compound **1**. As in the natural compound **1**, the mass spectrum of the synthetic compound **1** does not show additional fragment- or adduct-ions. The chromatographic analysis of a mixture of the crude extract and the synthetic **1** results in increased intensity of the compound peak in the crude extract (Fig. 3). It can therefore be concluded that the natural compound **1** has the same structure as the synthetic **1**.

Although Schmitz *et al.* [8] and Faulkner *et al.* [9] describe chloride as the predominant, if not exclusive natural counterion of halitoxin and the cyclostellettamines, we do not find any evidence for chloride as the counterion in the 3-alkyl pyridinium alkaloids contained in the crude extract of the Arctic sponge *Haliclona viscosa*. Instead, MALDI-TOF investigations of the crude extract show a different ion of yet unknown

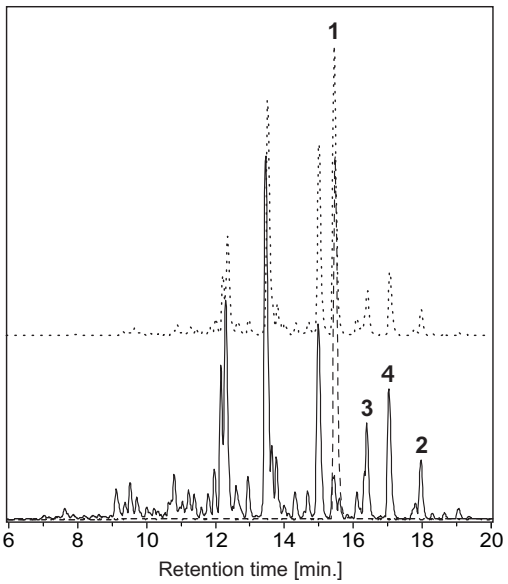


Fig. 3. Overlay of the HPLC-HRMS base peak chromatogram of the crude extract of a *Haliclona viscosa* sample from the year 2000 (black), the synthetic haliclocyclin C (**1**, dashed line), and a mixture of both (dotted line). The chromatogram peaks of haliclocyclin F (**2**), cyclostellettamine C (**3**), and viscosamine C (**4**) are indicated.

structure (unpublished results). This first report of haliclocyclin C (**1**) from a natural source completes the set of 3-APA oligomers with alkyl chains of equal length (C_{13}) isolated or identified in the Arctic sponge *Hali-*

clona viscosa. An interesting aspect is the progression of the bioactivity between the monomeric haliclocyclin C (**1**), the dimeric cyclostellettamine C (**3**), and the trimeric viscosamine (**4**) [5, 10]. The monomeric compound shows the highest, whereas the trimeric compound shows the lowest antimicrobial activity [11]. The cytotoxic activity in volumetric concentrations is almost identical for all three, while in molar concentrations viscosamine C (**4**) shows the highest and haliclocyclin C (**1**) the lowest cytotoxic activity [12]. While the monomer with a C_{14} alkyl chain, haliclocyclin F (**2**), also exists in the crude extract, the corresponding dimer and trimer with C_{14} alkyl chains, cyclostellettamine F and viscosamine F, have not been identified yet. On the other hand, a viscosamine in which at least one C_{14} alkyl chain is present was recently identified in *Haliclona viscosa* (unpublished results).

From the appearance of additional cyclic monomeric 3-alkyl pyridinium alkaloids we suggest that the to-date unnamed compound **2** should be renamed to haliclocyclin F; this suggestion follows the nomenclature of cyclostellettamines, where cyclostellettamine F contains two C_{14} alkyl chains [13]. Accordingly, compound **1** should be named haliclocyclin C (**1**) in analogy to cyclostellettamine C (**3**) and viscosamine C (**4**), both of which contain alkyl chains with 13 methylene groups.

Experimental Section

The sponge was collected in summer 2003 by the AWI scientific diving team using SCUBA in the Kongsfjord, Spitsbergen, at a depth of approximately 15 m. One part of the sponge was conserved in ethanol for taxonomic identification, which was performed by Wallie H. de Weerdt at the Zoölogisch Museum Amsterdam (voucher MAK301). The remaining sponge tissue was frozen at −20 °C and transported back to Bremerhaven where it was freeze-dried. Freeze-dried sponge tissue (4.18 g) was exhaustively extracted at room temperature with a 1 : 1 mixture of methanol and dichloromethane (4 × 80 mL).

The resulting crude extract was investigated by HR-LCMS on an Agilent 1100 HPLC system equipped with a Waters XTerra RP_{18} column (3.0 mm × 150 mm, 3.5 µm) applying an MeCN/H_2O (0.1 % HCOOH) gradient at 35 °C and a flow rate of 0.4 mL min^{-1}; 0 min: 20 % MeCN/80 % H_2O; 25 min: 55 % MeCN/45 % H_2O; 27 min: 100 % MeCN. UV spectra were recorded during HPLC analysis with a DAD (Agilent). Mass spectra were acquired on-line with a coupled Bruker Daltonics microTOF_{LC} mass spectrometer. The instrument was externally calibrated using sodium formate cluster. TLC used prefabricated, silica-coated aluminium sheets (Merck, silica 60) and a liquid phase of CH_2Cl_2/MeOH (9 : 1). NMR spectra were recorded on a Bruker Avance 250 MHz spectrometer at 298 K in [D_6]DMSO. Melting points were determined using a Kofler melting point apparatus and are uncorrected. Chemical shifts are quoted in ppm and are referenced to the appropriate solvent signal. FT-IR spectra were recorded on a Perkin-Elmer 1600 series spectrometer. Absorption maxima are reported in wave numbers, and the following abbreviations are used: s strong, m medium, w weak.

*Haliclocyclin C (**1**)*

The synthesis followed the method described in ref. [5]. This way **1** was obtained as a yellow solid in 60 % yield. – TLC: R_f = 0.25 (9 : 1 CH_2Cl_2/MeOH on Merck silica 60). – M. p.: 151 °C. – ^{1}H NMR (250 MHz, [D_6]DMSO): δ = 0.97 – 1.34 (m, 18 H, 9× CH_2), 1.67 – 1.82 (m, 2 H, 3-CH_2CH_2), 1.90 – 2.06 (m, 2 H, pyCH_2CH_2), 2.87 (t, J = 6.3 Hz, 2 H, 3-CH_2), 4.64 (t, J = 6.0 Hz, 2 H, pyCH_2), 8.12 (dd, J = 7.9 Hz, J = 6.2 Hz, 1 H, H5), 8.53 (d, J = 8.0 Hz, 1 H, H4), 8.98 (d, J = 6.0 Hz, 1 H, H6), 9.14 (s, 1 H, H2). – ^{13}C NMR (62.5 MHz, [D_6]DMSO): δ = 23.4, 25.5, 25.6, 25.8, 25.9, 26.2, 26.4, 26.6, 28.1, 28.8 (10× CH_2), 29.3 (3-CH_2CH_2), 30.5 (3-CH_2), 60.3 (CH_2N), 127.6 (C5), 142.3 (C6), 142.6 (C3), 143.8 (C2), 145.5 (C4). – IR (NaCl, cm^{-1}): ν = 3018 m, 2927 s, 2857 s, 1624 m, 1508 m, 1463 m, 1442 m, 1383 w, 1229 w, 1154 w, 931 w, 842 w, 814 w, 793 w, 717 w, 690 w. – HRMS ((+)-ESI): a) natural product: m/z = 260.2368 [M]$^+$, b) synthetic compound: m/z = 260.2360 [M]$^+$ (calcd. 260.2373 for $C_{18}H_{30}N^+$, [M]$^+$).

Acknowledgement

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[6] Viscosamine C (**4**) was originally published under the

name viscosamine [1]. It was recently renamed to viscosamine C [8] to account for the presence of additional viscosamines with different chain lengths. The name follows the nomenclature of the cyclostellettamines.

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[11] Antibacterial activity was tested against *Escherichia coli* tolC and *Staphylococcus aureus*. Results were obtained from an agar diffusion test with a test concentration of 20 μg. The diameter of growth inhibition is given in mm: **1** (19/16), **3** (12/11), and **4** (8/9).

[12] The cytotoxicity was tested against mouse fibroblasts L929. The IC_{50} values in $\mu g\,mL^{-1}$ are: **1** (1.2), **3** (1.0), and **4** (1.2). The molar activities (μM) are: **1** (4.62), **3** (1.92), and **4** (1.54).

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