New haliclamines E and F from the Arctic sponge *Haliclona viscosa*†

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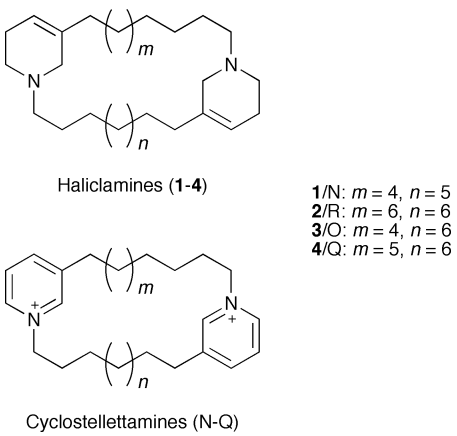
The Arctic sponge *Haliclona viscosa* is a rich source of 3-alkyl pyridinium alkaloids. Herein, we report the identification of two new haliclamines from the crude extract of this sponge. Due to the lack of sponge material, identification relied on HR-LCMS measurements and comparison with synthetic compounds.

Introduction

The sponge genus *Haliclona* comprises more than 400 species with a worldwide distribution and is a rich source of alkaloids, many of them with cytotoxic properties. Examples include the manzamines,[1] papuamines,[2] haliclonadiamines,[3] haliclamines,[4] halicyclamines,[5] and haliclonacyclamines.[6] The species *Haliclona viscosa* contains several types of 3-alkyl pyridinium alkaloids (3-APA) such as viscosaline,[7] the trimeric viscosamine,[8] the dimeric cyclostellettamines and haliclamines[9] and the first cyclic monomer of the 3-APA motif.[10] In our continuous search for bioactive compounds, we investigated the crude extract of a *H. viscosa* specimen collected in 2003. It contained two new 3-alkyl tetrahydropyridinium compounds, haliclamines E (**1**) and F (**2**), in addition to the main metabolites, haliclamines C (**3**) and D (**4**) (Scheme 1). Herein, we report the identification of the new compounds from the crude extract.



Scheme 1 Structural formulae of haliclamines E (**1**), F (**2**), C (**3**) and D (**4**), and cyclostellettamines N, R, O and Q.

Results and discussion

A freeze-dried specimen of *Haliclona viscosa*, collected off Blomstrandhalvøya in Kongsfjorden, Svalbard, by SCUBA diving in 2003 was exhaustively extracted with a 1:1 mixture of methanol/dichloromethane. The crude extract was subjected to an LCMS analysis. In addition to the main metabolites haliclamines C (**3**) and D (**4**),[9] it contained several unknown compounds. Since the sponge material was sparse and only a small amount of crude extract was available, further isolation of these compounds, followed by NMR spectroscopy, was not possible. However, the combined application of HPLC and HRMS indicated that two of the secondary metabolites were related to the haliclamines and the cyclostellettamines.

The high-resolution mass spectrum for compound **1** at a retention time (t_R, Fig. 1) of 27.2 min, obtained from ESI ionization, suggested the molecular formula $C_{29}H_{52}N_2$ (*m/z* 429.4217 $[M + H]^+$). The molecular weight was 14 mass units less than that of haliclamine C (**3**, *m/z* 443.4320 $[M + H]^+$; $t_R = 28.7$ min), indicating that one alkyl chain of compound **1** was by one methylene group shorter than haliclamine C (**3**). The atmospheric pressure ionization-collision induced dissociation-MS/MS (API-CID-MS/MS) spectrum of haliclamine C (**3**) showed two fragment peaks of *m/z* 208.210 and *m/z* 236.240, which correspond to a tetrahydropyridinium (THP) moiety connected to an alkyl chain of

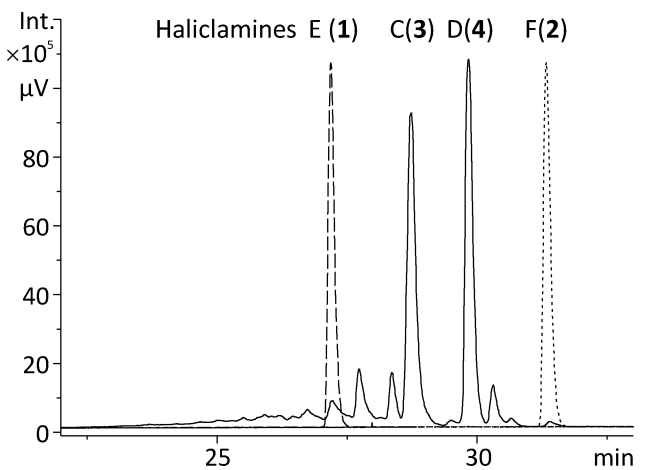


Fig. 1 Comparison of retention times of synthetic haliclamines E (**1**, dashed line) and F (**2**, dotted line) with the crude extract of *Haliclona viscosa* (continuous line).

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† Electronic supplementary information (ESI) available: MS spectra of the natural and the synthetic haliclamines; NMR spectra and shifts of the synthetic haliclamines; synthetic pathway of compounds **1**–**4**. See DOI: 10.1039/b904157e

Table 1 Comparison of mass spectrometric data of the naturally occurring and the synthetic haliclamine E (**1**). Masses were acquired with a Bruker micrOTOF mass spectrometer equipped with an ESI source

Ion[a]	Natural compound **1**	Synthetic compound **1**
$[M + H]^+$	$m/z = 429.4235$, $\Delta m = 7.5$ ppm	$m/z = 429.4217$, $\Delta m = 3.3$ ppm
$[M + 2H]^{2+}$	n.a.	$m/z = 215.2160$, $\Delta m = 10.1$ ppm
TFA salt $[M + H]^+$	n.a.	$m/z = 543.4123$, $\Delta m = 1.7$ ppm
fragment C_n	$m/z = 208.2118$, $\Delta m = 27.9$ ppm	$m/z = 208.2071$, $\Delta m = 5.5$ ppm
fragment C_m	$m/z = 222.2261$, $\Delta m = 20.1$ ppm	$m/z = 222.2220$, $\Delta m = 1.8$ ppm

[a] The doubly charged molecular ion originates from an experiment with a low voltage difference between capillary exit and skimmer. The singly charged fragments C_n/C_m consists of a tetrahydropyridinium moiety and a side chain of nine/ten methylene groups.

9 and 11 carbons, respectively.[9] The mass spectrum of compound **1** showed two similar fragments at m/z 208.2118 and m/z 222.2261, which suggested that **1** contains side chains of 9 and 10 methylene groups in addition to the two THP moieties. To add structural proof to this suggestion, the proposed compound **1** was synthesized by reduction of the corresponding cyclostellettamine.[11] The cyclostellettamine was prepared following a method described by Baldwin *et al.*[11] Subsequently, chromatographic and mass spectrometric data of the synthetic product were compared to those of the natural compound **1**.

The synthetic and the natural compound **1** eluted at the same retention time (Fig. 1). Both the molecular weight and the fragments of the synthetic product agreed with the natural compound. The synthetic product **1** showed a molecular mass of m/z 429.4217 ($[M + H]^+$), a doubly charged molecular ion at m/z 215.2160 and a peak at m/z 543.4123 resulting from salt formation with the TFA anion introduced during chromatographic purification (Fig. 2, Table 1). The mass spectrum of the synthetic compound also contained two peaks of lower intensity at m/z 208.2071 and m/z 222.2220 which correspond to the two fragments expected from THP moieties with C_9 and C_{10} alkyl chains each. Under API-CID-MS/MS (Skimmer-CID) conditions, these fragments became more pronounced whereas the doubly charged molecular ion and the TFA salt ion were eliminated (see Supporting Information for details†).

Compound **2** (m/z 471.4664 $[M + H]^+$; $t_R = 31.3$ min) was 14 mass units larger than haliclamine D (**4**, m/z 457.4470 $[M + H]^+$; $t_R = 29.8$ min) and suggested the molecular formula $C_{32}H_{58}N_2$. As haliclamine D (**4**) includes two chains of 10 and 11 methylene groups, respectively,[9] compound **2** was likely to contain chains of either 10 and 12 or chains of equal length of 11 methylene groups. Since the spectrum of the natural compound **2** also comprised a singly charged fragment at m/z 236.2422 it was concluded that the alkyl chains contained 11 methylene groups, and hence this compound was synthesized and compared. The synthetic product eluted at the same retention time (Fig. 1) and showed a similar mass spectrum as the natural compound **2** (Fig. 3, Table 2). It showed a molecular mass of m/z 471.4664 ($[M + H]^+$), a doubly charged molecular ion at m/z 236.2364 and a TFA adduct at m/z 585.4567. An API-CID-MS/MS experiment eliminated the TFA salt ion

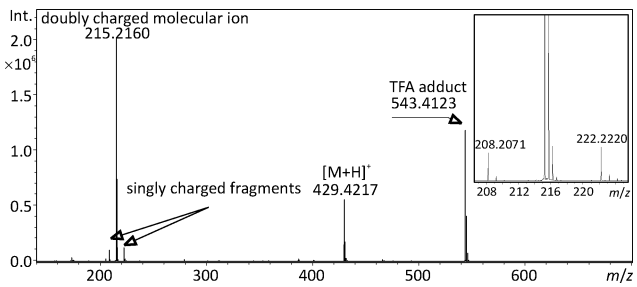

Fig. 2 ESI-TOF-MS spectrum of synthetic haliclamine E (**1**). The section shows a detail of the doubly charged molecular ion and the two singly charged fragments.

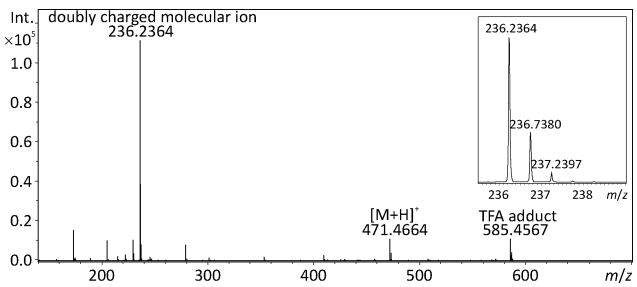

Fig. 3 TOF-MS spectrum of synthetic haliclamine F (**2**) under standard ESI conditions. The section shows a detail of the doubly charged molecular ion.

Table 2 Comparison of mass spectrometric data of the naturally occurring and the synthetic haliclamine F (**2**). Masses were acquired with a Bruker micrOTOF mass spectrometer equipped with an ESI source

Ion[a]	Natural compound **2**	Synthetic compound **2**
$[M + H]^+$	$m/z = 471.4710$, $\Delta m = 7.9$ ppm	$m/z = 471.4664$, $\Delta m = 1.8$ ppm
$[M + 2H]^{2+}$	n.a.	$m/z = 236.2364$, $\Delta m = 3.9$ ppm
TFA salt $[M + H]^+$	n.a.	$m/z = 585.4567$, $\Delta m = 5.8$ ppm
fragment $C_n = C_m$	$m/z = 236.2422$, $\Delta m = 21.0$ ppm	$m/z = 236.2355$, $\Delta m = 7.5$ ppm

[a] The doubly charged molecular ion originates from an experiment with low voltage difference between capillary exit and skimmer. The singly charged fragments C_n and C_m consist of a tetrahydropyridinium moiety and a side chain of 11 methylene groups, each.

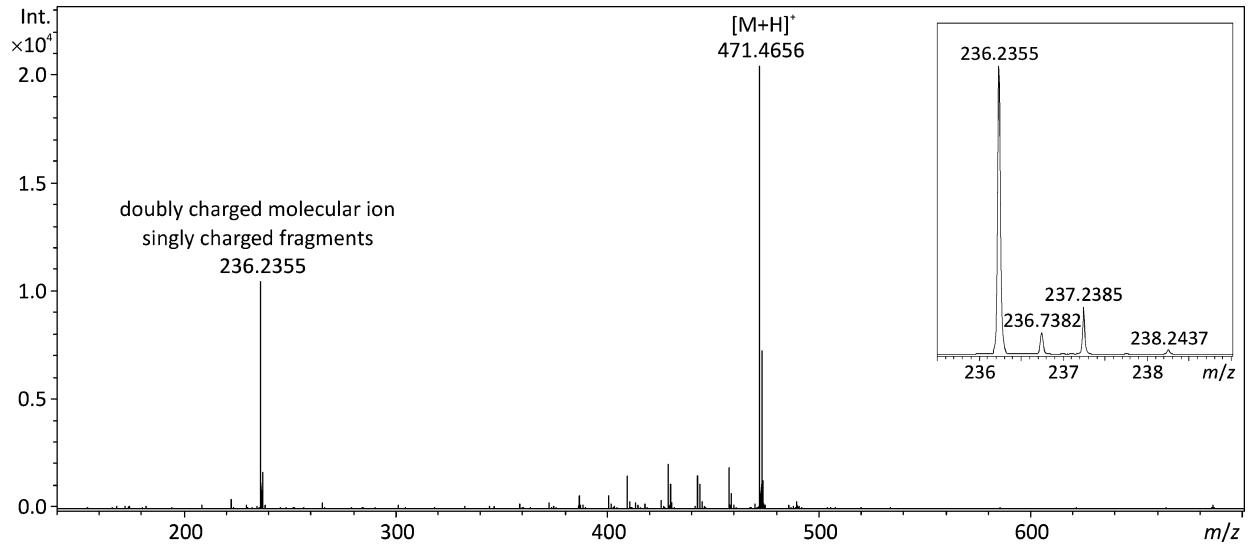

Fig. 4 ESI-TOF-MS spectrum of the synthetic haliclamine F (**2**) resulting from an API-CID-MS/MS experiment. The section shows a detail of the doubly charged molecular ion which is superimposed by the singly charged fragment.

and resulted in a peak at *m/z* 236.2355 that contained the isotopic pattern of the doubly charged molecular ion superimposed by the singly charged fragment (THP + C_{11} alkyl chain, Fig. 4).

Conclusions

Our investigations proved again that *Haliclona* sponges from Arctic waters are a rich source of interesting secondary metabolites. The two new members of the haliclamine family, the haliclamines E (**1**) and F (**2**), were directly identified from a sponge crude extract using a HR-LCMS method. In this context the identification was supported by the known biosynthetic origin of the 3-alkyl pyridinium alkaloids which are a common theme in sponges of the order Haplosclerida. Furthermore, a direct comparison was possible because the compounds were available through synthesis.

Experimental

Animal material

A specimen of *Haliclona viscosa* was collected off Blomstrandhalvøya by SCUBA diving in Kongsfjorden, Svalbard in June 2003. A voucher specimen is deposited at the Zoölogisch Museum, Amsterdam, The Netherlands (voucher reference MAK301, no ZMA registration number available yet). Samples of *H. viscosa* were divided into portions, immediately frozen after collection and kept at –20 °C until extraction.

Extraction and analysis of the natural compounds

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 (1.22 g, 29.2%) was investigated by HR-LCMS on an Agilent 1100 series instrument coupled to a micrOTOF$_{LC}$ mass spectrometer (Bruker Daltonics). The HPLC was equipped with a Waters XTerra MS C_{18} column (3 × 150 mm, 3.5 µm, 30 °C) and a MeCN/H_2O (5 mM NH_4OAc) gradient (30% MeCN/70% H_2O to 50% MeCN/50% H_2O in 30 min) at a flow rate of 0.4 mL min^{-1} was applied. UV spectra were recorded with a DAD at 260 nm. The mass spectrometer was equipped with an ESI source (capillary exit 100 V, skimmer 50 V).

Synthesis, purification and analysis of the synthetic compounds

All solvents were purified by distillation, with the exception of THF which was distilled from sodium/benzophenone under argon. A solution of the respective cyclostellettamine (0.13 mmol, cyclostellettamine N or R, Scheme 1) in 10 mL CH_2Cl_2/MeOH (1:1) was cooled to –40 °C and $NaBH_4$ (0.10 g, 2.63 mmol) was added. After stirring for two hours in the cold, the mixture was allowed to warm up to room temperature, 5 mL of 2 M NaOH solution were added and it was stirred for an additional 15 minutes. It was then poured onto 15 mL water and extracted with CH_2Cl_2 (3 × 10 mL). Drying of the combined organic extracts over $MgSO_4$ and removal of the solvent yielded the crude product (47 mg of **1**, 29 mg of **2**). The crude product was purified *via* preparative HPLC and yielded 32 mg (32%) of haliclamine E (**1**) and 20 mg (20%) of haliclamine F (**2**) as TFA salts.

Purification of the synthetic compounds was performed on a Jasco 1500 series HPLC system. It was equipped with a Prontosil Eurobond RP_{18} column (20 × 250 mm, 5 µm, 40 °C) applying a MeCN (0.1%TFA)/H_2O (0.1% TFA) gradient (30% MeCN/70% H_2O isocratic for 5 min; to 60% MeCN/40% H_2O in 30 min; isocratic for 5 minutes; to 100% MeCN in 10 min) with a flow rate of 8 mL min^{-1}. Analytical HPLC used a Kromasil RP_{18} column (4.6 × 250 mm, 5 µm, 40 °C), as before with a MeCN (0.1%TFA)/H_2O (0.1% TFA) gradient (from 100% H_2O to 80% MeCN/20% H_2O in 40 min; to 100% MeCN in 5 min) at a flow rate of 1 mL min^{-1}. UV spectra were recorded during HPLC analyses with a DAD (Jasco).

ESI mass spectra of the synthetic compounds were acquired using the micrOTOF$_{LC}$ mass spectrometer (Bruker Daltonics) in

direct injection mode and the same voltages as described for the natural compounds. For API-CID-fragmentation, the voltage of the capillary exit was raised to 180 V while the voltage of the skimmer 1 remained at 50 V. The MS system was externally calibrated in positive mode using sodium formiate cluster prior to every analysis.

Column chromatography was performed on silica gel 60 (Merck, particle size 0.04–0.063 mm). Aluminium plates precoated with Merck silica 60 were used for TLC. Compounds were visualized by UV irradiation (254 nm) or dyeing with $KMnO_4$ solution (1 g $KMnO_4$, 6.6 g K_2CO_3, 2 mL 5% NaOH solution in 100 mL H_2O). NMR spectra were recorded with a Bruker AM 300 (300 MHz) spectrometer. All experiments were performed at 300 K. 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.

Haliclamine E (1). White amorphous powder. TLC R_f: 0.43 (9:1 CH_2Cl_2/MeOH ($KMnO_4$)). IR (KBr, cm^{-1}): ν = 2932 m, 2860 m, 2623 w, 1685 s, 1465 w, 1406 w, 1198 s, 1162 s, 1133 s, 828 m, 797 m, 719 m. The compound does not show UV absorption. HRMS ((+)-ESI): m/z = 429.4217 (calcd. 429.4203 for $C_{29}H_{53}N_2^+$ $[M + H]^+$); m/z = 215.2160 (calcd. 215.2138 for $C_{29}H_{54}N_2^{2+}$ $[M + H]^{2+}$); m/z = 543.4123 (calcd. 543.4127 for $C_{29}H_{54}N_2^{2+} \times C_2F_3O_2^-$ $[M + H]^+$).

Haliclamine F (2). White amorphous powder. TLC R_f: 0.43 (9:1 CH_2Cl_2/MeOH ($KMnO_4$)). IR (KBr, cm^{-1}): ν = 2928 s, 2856 m, 1675 s, 1465 w, 1412 w, 1197 s, 1170 s, 1132 s, 828 w, 798 w, 720 m. The compound does not show UV absorption. HRMS ((+)-ESI): m/z = 471.4664 (calcd. 471.4673 for $C_{32}H_{59}N_2$); m/z = 236.2364 (calcd. 236.2373 for $C_{32}H_{60}N_2^{2+}$ $[M + H]^{2+}$); m/z = 585.4567 (calcd. 585.4601 for $C_{32}H_{59}N_2^{2+} \times C_2F_3O_2^-$ $[M + H]^+$).

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Notes and references

1 R. Sakai, T. Higa, C. W. Jefford and G. Bernardinelli, *J. Am. Chem. Soc.*, 1986, **108**, 6404–6405; R. Sakai, S. Kohmoto, T. Higa, C. W. Jefford and G. Bernardinelli, *Tetrahedron Lett.*, 1987, **28**, 5493–5496.
2 B. J. Baker, P. J. Scheuer and J. N. Shoolery, *J. Am. Chem. Soc.*, 1988, **110**, 965–966.
3 E. Fahy, T. F. Molinski, M. K. Harper, B. W. Sullivan, D. J. Faulkner, L. Parkanyi and J. Clardy, *Tetrahedron Lett.*, 1988, **29**, 3427–3428.
4 N. Fusetani, K. Yasumuro and S. Matsunaga, *Tetrahedron Lett.*, 1989, **30**, 6891–6894.
5 M. Jaspars, V. Pasupathy and P. Crews, *J. Org. Chem.*, 1994, **59**, 3253–3255.
6 R. D. Charan, M. J. Garson, I. M. Brereton, A. C. Willis and J. N. A. Hooper, *Tetrahedron*, 1996, **52**, 9111–9120; R. J. Clark, K. L. Field, R. D. Charan, M. J. Garson, I. M. Brereton and A. C. Willis, *Tetrahedron*, 1998, **54**, 8811–8826.
7 C. A. Volk and M. Köck, *Org. Biomol. Chem.*, 2004, **2**, 1827–1830.
8 C. A. Volk and M. Köck, *Org. Lett.*, 2003, **5**, 3567–3569.
9 C. A. Volk, H. Lippert, E. Lichte and M. Köck, *Eur. J. Org. Chem.*, 2004, 3154–3158.
10 C. Timm, C. Volk, F. Sasse and M. Köck, *Org. Biomol. Chem.*, 2008, **6**, 4036–4040.
11 J. E. Baldwin, D. A. James and V. Lee, *Tetrahedron Lett.*, 2000, **41**, 733–736.

