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Viscosamine: The First Naturally Occurring Trimeric 3-Alkyl Pyridinium Alkaloid†

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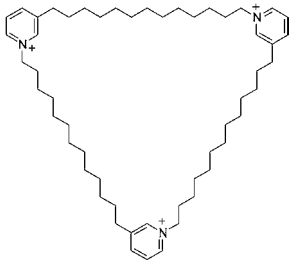
ABSTRACT



Pyridinium alkaloids are widely distributed in marine sponges of different genera. Chemical investigation of the Arctic sponge *Haliclona viscosa* led to the isolation of a new trimeric 3-alkyl pyridinium alkaloid (viscosamine). Trimers have not been described as natural products yet. The isolation and the structure elucidation of viscosamine are discussed in detail.

Sponges of the genus *Haliclona* are well-known to contain chemically diverse secondary metabolites with interesting biological activities, e.g., antifungal and cytotoxic activity.[1] Many of these compounds have been isolated and identified as alkaloids such as sarains, manzamines, papuamine, halicyclamines, haliclonacyclamines, and haliclonadiamines.[2] Structurally related metabolites were also described from other sponges such as *Callyspongia fibrosa*, *Echinochalina* sp., *Xestospongia* sp., and *Stelletta maxima*. Studies on *Haliclona* and related genera were mainly concentrated on tropical waters. Chemical investigations from Arctic waters were not reported in the literature yet.

In our search for bioactive secondary metabolites of marine organisms from temperate and polar waters, *Haliclona viscosa* was collected off the coast of Blomstrandhalvøya, near Hansneset, by SCUBA diving (15−25 m depth, June 2001) in the Kongsfjorden, which is located on the west coast of Svalbard at 79°N, 12°E. A voucher specimen is deposited under registration no. ZMA POR. 17009 at the Zoölogisch Museum, Amsterdam, The Netherlands.[3] Samples of *H. viscosa* were immediately frozen after collection and kept at −20 °C until extraction. Freeze-dried sponge tissue (14.38 g) was extracted at room temperature with a 1:1 mixture of

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(3) Sponge identification was kindly conducted by Wallie H. de Weerdt and Dr. Rob W. M. van Soest, Institute for Biodiversity and Ecosystem Dynamics (Zoological Museum), University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands.

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methanol/dichloromethane (3 × 1000 mL). The resulting greenish-colored crude extract (1.55 g) was partitioned between *n*-hexane (3 × 150 mL) and methanol (80 mL). The methanol extract was concentrated and further partitioned between ethyl acetate (3 × 150 mL) and water (80 mL), and finally the aqueous layer was extracted with 1-butanol (3 × 150 mL). The resulting 1-butanol phase (397.3 mg) was purified by preparative HPLC using 0.1% trifluoroacetic acid with acetonitrile/water gradients,[4] yielding 4 mg (0.03% of dry weight) of an unknown compound (**1**). The molecular formula of **1** was established by IR,[5] UV,[6] two-dimensional NMR,[7] and HR-MS techniques.[8] Table 1 summarizes the NMR chemical shift data of **1**.

Table 1. NMR Chemical Shift Data of Viscosamine (**1**) in DMSO-d_6[a]

no.[b]	$\delta(^{13}C)$[c] (**1**)	$\delta(^{1}H)$ (**1**)	$\delta(^{13}C)$ (**2**)	$\delta(^{1}H)$ (**2**)
1	(214)			
2	143.7	9.03	144.5	8.87
3	143.0		146.2	
4	144.9	8.48	146.2	8.45
5	127.4	8.08	128.3	8.02
6	142.1	8.94	142.5	8.81
7	60.4	4.56	62.7	4.61
8	30.2	1.90	31.9	2.00
9	*d*	1.20	26.4	1.23
10−16	*d*	1.20	range	range
17	*d*	1.25	range	1.26
18	29.3	1.63	30.8	1.73
19	31.2	2.78	33.0	2.89

[a] Chemical shifts δ are given in parts per million (ppm). ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.50 ppm). For comparison, chemical shifts of cyclostellettamine C (**2**)[2k] are given in the last two columns of the table. All NMR correlation data are given in Supporting Information in detail. [b] Since **1** is C_3 symmetric, 1 stands for 1, 1′, and 1″; 2 for 2, 2′, and 2″, etc. [c] For position no. 1, $\delta(^{15}N)$ is given. ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [d] It was not possible to assign a single chemical shift value.

The proton chemical shifts as well as the characteristic $^{1}H,^{1}H$ coupling constants indicated a 1,3-disubstituted pyridinium ring system. The pyridinium moiety is further supported by the $^{1}J_{CH}$ coupling constants of the ring carbons (170−190 Hz, see Table 1 in Supporting Information), as well as the proton and carbon chemical shifts of the methylene group adjacent to the nitrogen atom (>4.5 ppm, >60 ppm). Another hint is the ^{15}N chemical shift of 214 ppm, which corresponds to a pyridinium nitrogen.[9] Since more than 50% of the proton signals appear at one resonance frequency, the complete constitutional assignment cannot be carried out by NMR methods. The edited HSQC showed that all aliphatic carbons are methylene groups. The NMR data suggested that the pyridinium moieties are connected through long alkyl chains. These kinds of compounds were already published by Fusetani et al. almost 10 years ago, the so-called cyclostellettamines (**2**, cyclostellettamine C), which are dimeric 3-alkyl pyridinium alkaloids connected by C_{12} to C_{14} alkyl chains.[2k]

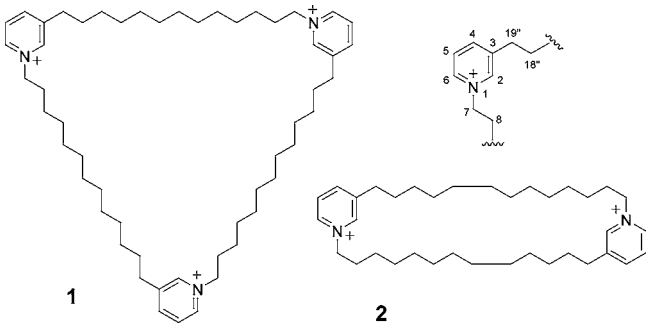

Figure 1. Viscosamine (**1**) from *Haliclona viscosa* and cyclostellettamine C (**2**).

High-resolution ESI-MS of **1** showed only doubly and triply charged ions (see Table 2). The mass of the triply charged ion (m/z = 260.2) suggested a trimeric 3-alkyl pyridinium alkaloid with the same alkyl chain lengths as

Table 2. HR-ESIMS Data Obtained for **1** (M = 780)

obsd mass	charge[a]	calcd mass[b]	molecule ion
446.8403	2	893.6985	$C_{56}H_{90}N_3O_2F_3$
419.8607	2	839.7268	$C_{56}H_{93}N_3O_2$
389.8506	2	779.7010	$C_{54}H_{89}N_3$
260.2313	3	780.7135	$C_{54}H_{90}N_3$

[a] Due to the splitting of the isotope peaks. [b] Mass deviation for: 893.6985, Δm = −17.9 mDa; 839.7268, Δm = −6.4 mDa; 779.7010, Δm = −6.0 mDa; 780.7135, Δm = −19.6 mDa.

cyclostellettamine C (**2**). Cyclostellettamine C ($C_{36}H_{60}N_2$) has a molecular weight of 520.4, which would appear at m/z = 260.2 in the MS spectrum because of the double charge. The singly charged mass ion of **2** is observed at m/z = 519.4

(4) For HPLC analysis, samples were injected into a HPLC system (JASCO) equipped with a light scattering detector SEDEX 75 (Sedere). The analytical columm (4.6 × 250 mm, 5 μm) and the separation columm (16 × 250 mm, 10 μm) were prefilled with Kromasil RP-18 (Knauer GmbH). Separation was achieved by applying a gradient from 5% acetonitrile (containing 0.1% trifluoroacetic acid) to 35% acetonitrile over a period of 40 min. For extraction, solvents were distilled prior to use, and gradient-grade solvents were used for chromatographic applications.

(5) IR spectra (KBr) were recorded on a Bruker EQUINOX 55 spectrometer (Bruker Optik GmbH). IR (KBr) of **1**: ν_{max} 2927, 2855, 1685 and 1202 cm^{-1}.

(6) UV spectra (MeOH) were recorded on a UVIKON 810P spectrometer (KONTRON). UV (MeOH) of **1**: λ_{max} 266.8 and 203.4 nm.

(7) ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 and Avance 500 spectrometers (Bruker BioSpin GmbH). A 4 mg sample of **1** in 0.6 mL DMSO-d_6 was used for the NMR measurements. All NMR experiments were measured at 300 K.

(8) Mass spectral analyses were performed on ESI-TOF (LCT, Micromass) and Q-TOF (Micromass) MS spectrometers.

(9) (a) Berger, S.; Braun, S.; Kalinowski, H.-O. *^{15}N NMR-Spektroskopie*; Georg Thieme Verlag: Stuttgart; 1992, Vol. 2. (b) $\delta(^{15}N)$ of the pyridinium-*N* is about 210 ppm in comparison to pyridine-*N* 317 ppm and pyrrole-*N* 149 ppm.

due to a Hofmann-type fragmentation. The observation of mass peaks for these kind of compounds with one mass number less than the actual molecular weight was already described by Faulkner et al. in 1993.[10]

Also the other three MS signals are in agreement with the trimeric structure of viscosamine (**1**) (see Table 2). The MS peak at $m/z = 389.8$ represents the doubly charged molecule ion; the mass is one less than expected due to a Hofmann-type fragmentation (for more details see Table 2 in Supporting Information). All four MS/MS traces ($m/z = 446.8$, 419.8, 389.8, and 260.2) showed a singly charged ion at $m/z = 260.2$, as was also observed for cyclostellettamine C (**2**).[2k] Therefore, the new alkaloid viscosamine (**1**) was identified as the trimeric derivative of the known dimeric compound cyclostelletamine C (**2**). The symmetry of the NMR spectrum indicated the C_3 symmetric structure of **1**.

Trimeric compounds are relatively rare in nature; usually only dimeric or polymeric natural products were isolated. Viscosamine (**1**) is the first trimeric 3-alkyl pyridinium compound from a marine environment.

(10) Davies-Coleman, M. T.; Faulkner, D. J.; Dubowchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925−5930.

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Supporting Information Available: Tables with extended NMR and MS data and ^{1}H NMR, HRESI, and MS/MS spectra for Viscosamine (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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