Cyclohaliclonamines A−E: Dimeric, Trimeric, Tetrameric, Pentameric, and Hexameric 3-Alkyl Pyridinium Alkaloids from a Marine Sponge *Haliclona* sp.

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A mixture of cyclohaliclonamines A−E (**1**−**5**), novel dimeric, trimeric, tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids, was obtained from an Okinawan sponge *Haliclona* sp. Cyclohaliclonamines C−E are the first tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids from natural sources. The structure determination of cyclohaliclonamines is discussed in detail.

3-Alkylpyridine alkaloids are a well-known family of marine natural products, including navenones,[1] halitoxins,[2] niphatynes,[3] theonelladins,[4] ikimins,[5] xestamines,[6] niphatoxins,[7] niphatesines,[8] haminols,[9] cyclostellettamines,[10,11] untenines,[12] and viscosamine.[13] Although dimeric and trimeric 3-alkylpyridinium alkaloids such as cyclostellettamines[10] and viscosamine[13] have been isolated from marine sponges, no tetrameric, pentameric, or hexameric 3-alkylpyridinium alkaloids have been isolated. We report here the structure determination of cyclohaliclonamines A−E (**1**−**5**), dimeric, trimeric, tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids from the Okinawan sponge *Haliclona* sp. (Chart 1).

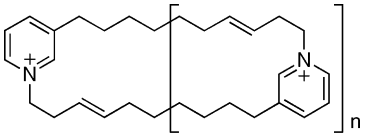
The Okinawan sponge *Haliclona* sp. (95.0 g), collected at Bise, Okinawa Prefecture, Japan, was extracted with MeOH. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. Water-soluble materials were extracted with *n*-BuOH. The material obtained from the *n*-BuOH portion was subjected to fractionation guided by toxicity against brine shrimp with column chromatography (ODS silica gel, MeOH−H_2O) and reversed-phase HPLC (Develosil ODS-HG-5, MeOH−H_2O−TFA) to give cyclohaliclonamine (1.5 mg).

The ^{1}H NMR analysis of this cyclohaliclonamine showed patterns very characteristic of a 3-alkylpyridinium compound (Table 1), including four aromatic hydrogen signals at δ 8.85 (br s, H-2), 8.75 (d, J = 6.0 Hz, H-6), 8.43 (d, J = 7.9 Hz, H-4), and 7.98 (dd, J = 7.9, 6.0 Hz, H-5) as well as aliphatic hydrogen signals at δ 4.60 (t, J = 6.8 Hz, H-7) and 2.84 (t, J = 7.8 Hz, H-16).[10,11,13]

A detailed analysis of the COSY spectrum of cyclohaliclonamine allowed the construction of four partial structures, C2−C6, C7−C9, C10−C12, and C13−C16, as shown in Figure 1. The connectivities of these four partial structures were clarified by HMBC correlations: H16/C3, H15/C3, H2/C3, H5/C3, H2/C7, H6/C7, H7/C2, H7/C6, H7/C9, H8/C9, H8/C10, H11/C9, and H11/C10. Although no additional connectivities were obtained from the NMR analysis because of overlapping H-12 and H-13 resonances, C12 and C13 in cyclohaliclonamine should be connected, considering its ^{1}H NMR and ^{13}C NMR spectra (Table 1). The *E*-geometry of the double bond in cyclohaliclonamine was deduced from the ^{13}C chemical shift values of the allylic methylenes (δ 35.5 and 33.6).[12]

To confirm the molecular formula of cyclohaliclonamine, the ESIMS was measured. Although the HPLC analysis of this fraction exhibited a single peak, the isotope peaks of the expected molecular ion peak at *m*/*z* 216.1 were complicated because of the multicharged molecular ion. In addition, the high-resolution ESIMS showed singly, doubly, triply, quadruply, and quintuply charged ions arising from a Hofmann-type fragmentation of the corresponding oligomeric 3-alkylpyridinium alkaloids (Table 2). These results revealed the presence of dimeric, trimeric, tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids. Faulkner et al. reported the

Chart 1



1 cyclohaliclonamine A n=1
2 cyclohaliclonamine B n=2
3 cyclohaliclonamine C n=3
4 cyclohaliclonamine D n=4
5 cyclohaliclonamine E n=5

Table 1. NMR Data for Cyclohaliclonamine in CD_3OD

no.	^{1}H (ppm)[a]	^{13}C (ppm)[b]	HMBC
1			
2	8.85 br s	145.2	C3, 7
3		145.6	
4	8.43 (7.9)[c]	146.6	C2, 6
5	7.98 (7.9, 6.0)	128.7	C3, 6
6	8.75 (6.0)	143.4	C4, 5, 7
7	4.60 (6.8)	62.4	C2, 6, 8, 9
8	2.66 m	35.5	C9, 10
9	5.43 m	124.7	
10	5.43 m	137.2	
11	1.94 m	33.6	C9, 10
12	1.30 m	30.3[d]	
13	1.30 m	30.1[d]	
14	1.37 m	30.0[d]	
15	1.68 m	31.7	C3
16	2.84 (7.8)	33.5	C3, 15

[a] Recorded at 500 MHz. [b]Recorded at 125 MHz. [c]Coupling constants (Hz) are in parentheses. [d]Assignments with the same superscript may be interchanged.

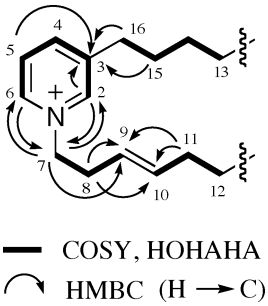


Figure 1. Partial structure of **1**, based on 2D NMR correlations.

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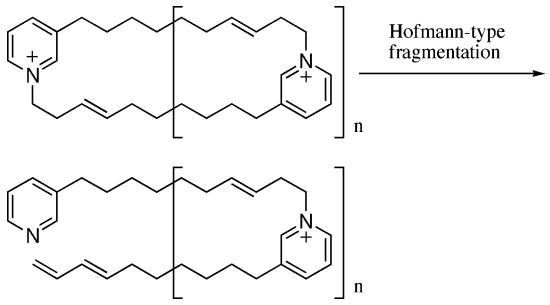
Table 2. HRESIMS Data Obtained for Cyclohaliclonamines A−E (**1**−**5**)



compound	*n*	obsd mass	charge[a]	calcd mass[b]	molecular formula
cyclohaliclonamine A	1	431.3398	1	431.3426	$C_{30}H_{35}N_2$
cyclohaliclonamine B	2	323.7590	2	647.5178	$C_{45}H_{53}N_3$
cyclohaliclonamine C	3	287.9036	3	863.6931	$C_{60}H_{71}N_4$
cyclohaliclonamine D	4	269.9707	4	1079.8683	$C_{75}H_{109}N_5$
cyclohaliclonamine E	5	259.2164	5	1296.0435	$C_{90}H_{131}N_6$

[a] Due to the splitting of the isotope peaks. [b]Mass deviation for 431.3426, $\Delta m = -2.8$ mmu; 647.5178, $\Delta m = -0.2$ mmu; 863.6931, $\Delta m = 17.7$ mmu; 1079.8683, $\Delta m = 14.5$ mmu; 1296.9671, $\Delta m = 38.5$ mmu.

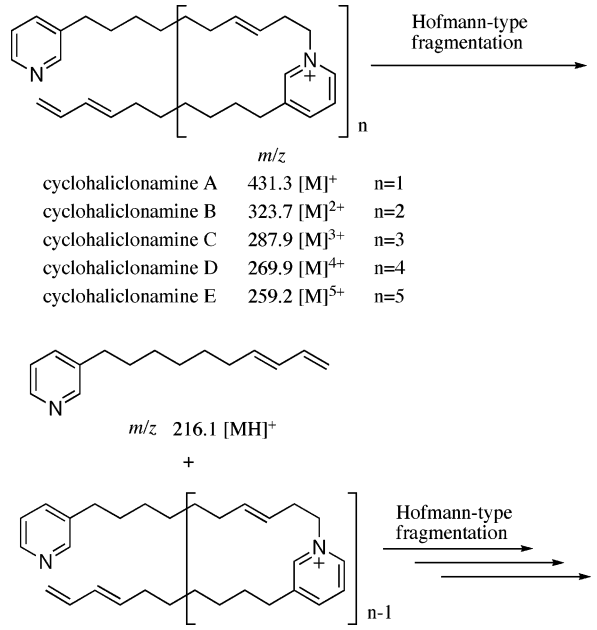


Figure 2. Proposed mass fragments (MS/MS analysis) of cyclohaliclonamines A−E (**1**−**5**).

observation of mass peaks due to a Hofmann-type fragmentation for cyclic pyridinium alkaloids in 1993.[14] Separation of cyclohaliclonamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) failed with normal-phase, reversed-phase, and size-exclusion chromatography. Berlinck reported isolation of cyclostellettamines G−I, K, and L, dimeric 3-alkylpyridinium alkaloids, but could not separate cyclostellettamines G−I, K, and L.[15]

The molecular formulas of cyclohaliclonamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) were established as $(C_{30}H_{44}N_2)^{2+}$, $(C_{45}H_{66}N_3)^{3+}$, $(C_{60}H_{88}N_4)^{4+}$, $(C_{75}H_{110}N_5)^{5+}$, and $(C_{90}H_{132}N_6)^{6+}$, respectively, on the basis of ESIMS data with ion peaks at *m*/*z* 431.3398 $[M]^+$, 323.7590 $[M]^{2+}$, 287.9036 $[M]^{3+}$, 269.9707 $[M]^{4+}$, and 259.2164 $[M]^{5+}$, arising from a Hofmann-type fragmentation (Table 2). The five MS/MS traces (*m*/*z* 431.3, 323.7, 287.9, 269.9, and 259.2) showed the same singly charged ion at *m*/*z* 216.1 (see Figure 2 and Supporting Information). When the collision energy of the MS/MS trace was low, no mass fragments arising from a Hofmann-type fragmentation were observed, indicating that these mass peaks on ESIMS of cyclohaliclonamine are not the fragment ion peaks of bigger oligomers, but Hofmann-type fragment peaks of dimeric, trimeric, tetrameric, pentameric, and hexameric pyridinum alkaloids. These results and the NMR data suggested that **1**, **2**, **3**, **4**, and **5** were dimeric, trimeric, tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids with C_{10} alkyl chains, respectively. Thus, the structures of cyclohaliclonamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) were determined to be those shown in formulas **1**−**5**, and the natural cyclohaliclonamine is a mixture of cyclohaliclonamines A−E (**1**−**5**).[16]

The cyclohaliclonamine mixture showed a toxicity against brine shrimp with an LD_{50} of 65 μg/mL.

These kinds of compounds were isolated from marine sponges. Fusetani et al.[10,11] and Berlinck et al.[15] reported cyclostellettamines that are dimeric 3-alkylpyridinium alkaloids connected by C_{10} to C_{14} alkyl chains. In addition, Kock and Volk reported the isolation and structure determination of a trimeric 3-alkylpyridinium alkaloid, viscosamine.[13] A tetrameric 3-alkylpyridinium alkaloid was synthesized by Davies-Coleman et al.[14] Although dimeric, trimeric, and polymeric[2,14] 3-alkylpyridinium alkaloids have been isolated from marine organisms, cyclohaliclonamines C (**3**), D (**4**), and E (**5**) represent the first tetrameric, pentameric, and hexameric 3-alkylpyridinium alkaloids.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks ($\delta_H = 3.31$ ppm and $\delta_C = 49.5$ ppm in methanol-d_4). High-resolution mass spectra (HRMS) were obtained on a PE Sciex QSTAR mass spectrometer. Column chromatography was performed on ODS gel (Nacalai Tesque, Cosmosil 75C_{18}-OPN). Reversed-phase high-performance liquid chromatography (HPLC) was carried out on a Develosil ODS-HG-5 column (Nomura Chemical Co., Ltd).

Biological Material. The sponge material was collected by hand using scuba at a depth between 1 and 2 m off Bise, Okinawa, Japan, in January 2005. The *Haliclona* sp. was identified by Dr. John N. A. Hooper (Queensland Museum, Australia) and corresponds to Queensland Museum voucher number QM G324174.

Extraction and Separation. The frozen sponge materials of *Haliclona* sp. (95 g) were extracted with MeOH at room temperature. The extracts were concentrated and partitioned between EtOAc and H_2O. The H_2O-soluble materials were partitioned with *n*-BuOH and H_2O. The *n*-BuOH-soluble material (429 mg) was first separated by column chromatography on ODS (6.0 g) using 40% MeOH, 60% MeOH, 80% MeOH, and MeOH. The fraction (50 mg) eluted with 80% MeOH was subjected to HPLC [Develosil ODS-HG-5 (250 × 20 mm); flow late 5 mL/min; detection, UV

215 nm; solvent 70% MeOH/0.1% trifluoroacetic acid] to give cyclohaliclonamines (1.5 mg, retention time 6 min).

Brine Shrimp Toxicity Assay. The screening for brine shrimp toxicity was performed using a slight modification of the original method.[10] Samples were dissolved in MeOH. Appropriate amounts of solution were transferred to 1.0 cm disks of filter paper. The disks were dried in vacuo for 1 h. Control disks were prepared using only MeOH. Approximately 10 hatched brine shrimp were transferred to each sample vial, and artificial seawater was added to make 1 mL. After 24 h at 25 °C, the numbers of living and dead brine shrimp were determined. The activity is expressed in terms of LD_{50} to account for a significant number of brine shrimp that were visually affected and their movements inhibited. Berberine chloride showed toxicity against brine shrimp with an LD_{50} of 22.5 μg/mL[17]

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Supporting Information Available: Spectral data of cyclohaliclonamines are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Sleeper, H. L.; Fenical, W. *J. Am. Chem. Soc.* **1977**, *99*, 2367−2368.
(2) Schmitz, F. J.; Hollenbeak, K. H.; Campbell, D. C. *J. Org. Chem.* **1978**, *43*, 3916−3922.
(3) Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 2467−2468.
(4) Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1989**, *30*, 4833−4836.
(5) Carroll, A. R.; Scheuer, P. J. *Tetrahedron* **1990**, *46*, 6637−6644.
(6) Sakemi, S.; Totton, L. E.; Sun, H. H. *J. Nat. Prod.* **1990**, *53*, 995−999.
(7) Talpir, R.; Rudi, A.; Ilan, M.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 3033−3304.
(8) Kobayashi, J.; Zeng. C. M.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Mikami, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1291−1294.
(9) Spinella, A.; Alvarez, L. A.; Passeggio, A.; Cimino, G. *Tetrahedron* **1993**, *49*, 1307−1314.
(10) Fusetani, N.; Asai, N.; Matsunaga, S. Honda, K.; Yasumuro, K. *Tetrahedron Lett.* **1994**, *35*, 3967−3970.
(11) Oku, N.; Nagai, K.; Shindoh, N.; Terada, Y.; van Soest, R. W. M.; Matsunaga, S.; Fusetani, N. *Bioorg. Med. Chem. Lett.* **2004**. *14*, 2617−2620.
(12) Wang, G.-Y.-S.; Kuramoto, M.; Uemura, D.; Yamada, A.; Yamaguchi, K.; Yazawa, K. *Tetrahedron Lett.* **1996**, *37*, 1813−1816.
(13) Volk, C. A.; Kock, M. *Org. Lett*. **2003**, *5*, 3567−3569.
(14) Davies-Coleman, M. T.; Faulkner, D. J.; Dubowchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925−5930.
(15) De Oliveria, J. H. H. L.; Grube, A.; Kock, M.; Berlinck, R. G. S.; Ferreira, M. A. G.; Hajdu, E. *J. Nat. Prod.* **2004**, *67*, 1685−1689.
(16) Estimation of molecular weight distribution of cyclohaliclonamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) failed with size-exclusion chromatography and SDS-PAGE electrophoresis.
(17) Meyer, B. N.; Ferringni, J. E.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; Mclaughlin, J. *Planta Med.* **1982**, *45*, 31−34.

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