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Haliclonin A, a New Macrocyclic Diamide from the Sponge *Haliclona* sp.

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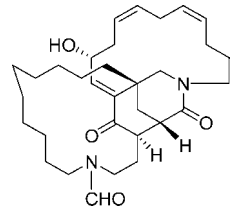
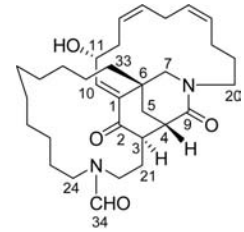
ABSTRACT



Haliclonin A (1), a macrocyclic diamide of a novel skeletal class, was isolated from the marine sponge *Haliclona* sp. collected from Korean waters. The structure of this compound was determined using a combination of spectroscopic and chemical analyses. The new compound exhibited moderate cytotoxicity and antibacterial activity against diverse microbial strains.

Macrocyclic diamine metabolites derived from 3-alkylpyridine dimers have been isolated from a variety of marine sponges.[1,2] The most familiar example may be sarain A, a heptacyclic diamine from *Reniera* (*Haliclona*) *sarai*. In addition, manzamines from *Haliclona* sp. alkaloids with potent bioactivity against various infectious diseases possess macrocyclic portions which are thought to have originated from a precursor of the same class.[3−5] The structural uniqueness and potent bioactivity of these compounds attract considerable interest for both their biomedical and synthetic aspects.[6−8]



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During the course of our search for bioactive compounds from marine sponges of Korea, we encountered a specimen of *Haliclona* sp. whose crude extract exhibited moderate toxicity toward brine shrimp larvae (LC_{50} 260 ppm). The bioactive constituent was isolated following a bioassay-

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guided separation using diverse chromatographic methods. We report herein the structure of haliclonin A (**1**), a cytotoxic and antimicrobial tetracyclic diamide of a new skeletal class.

Compound **1** was obtained as a colorless gum, $[\alpha]_D^{20}$ −23.6 (*c* 0.14, MeOH). The molecular formula of compound **1**was deduced as $C_{32}H_{48}N_2O_4$ by high-resolution fast atom bombardment mass spectra (HRFABMS) analysis (+0.3 mmu) at *m*/*z* 525.3696 $[M + H]^+$ (calcd for $C_{32}H_{49}N_2O_4$, 525.3692). However, the NMR data for this compound were far more complex than expected on the basis of the mass data. The ^{13}C NMR spectra exhibited 61 carbon signals with various intensities. The ^1H NMR spectra also contained significantly more proton signals than anticipated. A detailed examination of the NMR data revealed that most of the proton and carbon signals were paired to each other, implying that compound **1** was either a mixture of two very similar compounds or conformers with a ratio determined to be 3:2 based on the integration of downfield proton signals.

Given this preliminary information, the structure of **1** was determined mainly for the major constituent (**1a**). The ^{13}C NMR data of **1a** displayed signals of carbonyl carbons at δ 202.4, 170.8, and 162.9, indicating an α,β-unsaturated ketone and two amides, considering the molecular formula (Table 1). This interpretation was consistent with the IR absorption bands at 1680 (sh) and 1650 cm^{-1} and the UV maximum at 286 nm. The presence of a downfield signal at δ 6.74 (1 H, br d, J = 10.6 Hz) in the ^1H NMR spectra revealed that a trisubstituted double bond was attached to a carbonyl group.

With the aid of ^1H COSY and gHSQC data, a cyclohexanone moiety containing an *exo*-double bond was readily deduced and confirmed by the long-range correlations between the carbons at δ 202.4, 138.7, 47.8, 41.4, 39.3, and 31.9 with the protons attached to these carbons in the gHMBC data (Table 1). The 2-D NMR analyses were extended to the neighboring signals, including the amide carbon at δ 170.8 and a methylene at δ 61.1, to construct another six-membered lactam moiety. Thus, an isoquinoline-related 3-azabicyclo[3.3.1]nonane ring system was defined to possess a ketone and an amide group. The attachment of an ethylene group to the α-position of the ketone was also determined by ^1H COSY data (Figure 1, (a)).

Furthermore, ^1H COSY and TOCSY data revealed the presence of a long proton spin system possessing an olefinic proton at δ 6.74 and methylene protons at δ 4.24 and 2.49 at the termini. The placement of a secondary hydroxyl group

Table 1. NMR Assignments for Compound **1**[a]

no.	δ_H	δ_C	HMBC
1[b]		138.7	
1[c]		138.1	
2[b]		202.4	
2[c]		201.5	
3[b]	2.71, m	47.84	C-2, 4, 21, 22
3[c]	2.71, m	47.78	
4[b]	2.77, m	41.4	C-2, 3, 5, 6, 9, 21
4[c]	2.69, br s	41.5	
5[b]	2.21, br d, (11.7); 1.69, m	31.9	C-1, 4, 6, 7, 9, 33
5[c]	2.25, m; 1.72, m	31.6	
6		39.3	
7	3.24, m; 3.22, m	61.1	C-1, 5, 6, 9, 20, 33
9		170.8	
10[b]	6.74, br d, (10.6)	142.8	C-1, 2, 6, 11, 12
10[c]	6.78, br d, (10.8)	143.6	
11	4.67, m	67.3	C-1, 10, 12, 13
12	2.52, m; 2.26, m	34.2	C-10, 11, 13, 14
13	5.68, dt, (10.4, 8.0)	123.9	C-11, 12, 15
14	5.81, m	131.4	C-12, 15
15	2.86, m; 2.49, m	25.9	C-13, 14, 16, 17
16	5.32, ddd, (10.8, 10.8, 4.8)	128.1	C-15, 17, 18
17	5.39, m	127.1	C-15, 16, 18
18	2.04, m; 1.81, m	24.0	C-16, 17, 19, 20
19	1.60, m; 1.40, m	27.2	C-18, 20
20	4.24, m; 2.49, m	45.4	C-7, 9, 18, 19
21[b]	2.12, m; 1.90, m	31.3	C-2, 3, 4, 22
21[c]	2.10, m; 1.79, m	33.7	
22[b]	3.56, dt, (3.6, 12.6) 3.51, dt, (3.6, 12.6)	42.0	C-3, 21, 24, 34
22[c]	3.61, dt, (3.6, 12.6) 3.42, dt, (3.6, 12.6)	46.6	
24[b]	3.29, dt, (7.2, 13.5); 3.21, m	49.2	C-22, 25, 26, 34
24[c]	3.48, m; 3.16, m	43.3	
25	1.58, m; 1.48, m	27.6	C-24
26	1.39, m; 1.33, m	25.1	C-24
27∼30	1.30 ∼ 1.23	27.9∼27.2	
31	1.33, m	25.4	C-33
32	1.35, m	29.0	C-33
33	2.14, m; 1.34, m	36.8	C-1, 5, 6, 7, 31, 32
34[b]	8.02, s	162.9	C-22, 24
34[c]	8.11, s	162.8	

[a] Data were obtained in $CDCl_3$ solution. [b,c] Denote chemical shifts for the major (**1a**) and minor rotational isomer (**1b**), respectively.

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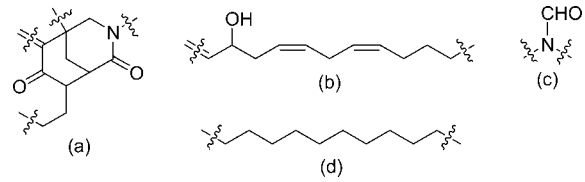

Figure 1. Partial structures of compound **1a**.

and two double bonds in the middle of the chain was confirmed by gHMBC data (Figure 1, (b)). Chemical shifts of an isolated proton at δ 8.02 and its attached amide carbon at δ 162.9 indicated the presence of a foramide group (Figure 1, (c)). Consideration of the molecular formula suggested that the remaining portion of the molecule consisted of 10 methylene carbons. However, most of these carbons and their protons were not accurately assigned because of the severe overlapping of the proton signals, as well as interference from the carbon signals of the minor constituent (**1b**) in the NMR data; a 10-methylene linear chain possessing methylenes at δ_C 49.2 (δ_H 3.29 and 3.21) and 36.8 (δ_H 2.14 and 1.34) at the termini (Figure 1, (d)) was illustrated on the basis of combined ^{1}H COSY and TOCSY data in which these terminal protons correlated with those in upfield.

The connectivity of the partial structures was determined by gHMBC experiments. Long-range couplings of the olefinic proton at δ 6.74 in (b) with carbons at δ 202.4 and 39.3 of (a) secured the presence of an enone moiety, thereby connecting these partial structures. Similarly, long-range couplings between the methylene protons at δ 4.24 and 2.49 with the lactam carbons at δ 170.8 and 61.1 allowed us to construct another linkage between (a) and (b). Mutual couplings among the carbons at δ 162.9, 49.2, and 42.0 and their attached protons connected (a), (c), and (d), thus forming a tertiary foramide group. Finally, the linkage of (d) at the quaternary center of (a) was established by the long-range couplings between the methylene protons at δ 2.14 and 1.34 at the terminus of the former and the carbons at δ 138.7, 61.1, 39.3, and 31.9 of the latter. Thus, the planar structure of **1a** was unambiguously determined to be a tetracyclic diamide.

An examination of the literature revealed that the presence of two macrocyclic skeletons, as well as the locations of the two nitrogen atoms in the molecule, is reminiscent of sarain A and other bis-alkylpyridinium-derived compounds from sponges.[1] To the best of our knowledge, however, the structure of **1a** is unprecedented for not only its 3-azabicyclononane framework but also the presence of an enone and two amide functionalities.

The stereochemistry at asymmetric carbon centers was determined by NOESY experiments. A simple three-dimensional model demonstrated that in order to form a 3-azabicyclo[3.3.1]nonane system as in **1a**, the cyclohexanone must be connected axially to the lactam ring. This was evidenced by a series of NOESY cross peaks at H-5eq (δ 2.21)/H-22 (δ 3.56), H-4/H-22 (δ 3.56), and H-4/H-21 (δ 2.12) for the orientation at C-4, and those at H-4/H-5ax (δ 1.69), H-5ax/H-7, H-5ax/H-33 (δ 1.34), and H-7/H-33 (δ 1.34 and 2.14) for the orientation at C-6, respectively (Figure 2). Similarly,

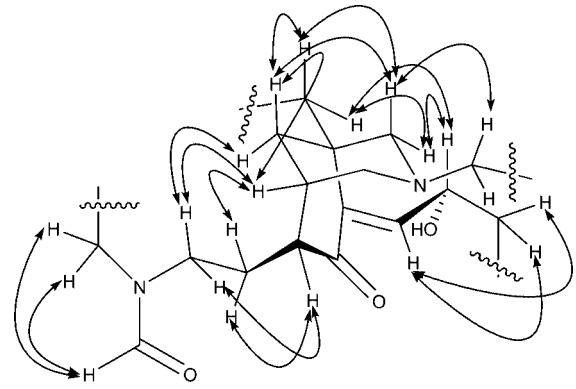

Figure 2. Selected NOESY correlations for compound **1a**.

the equatorial orientation of H-3 to the cyclohexanone ring was assigned by its cross peaks with H-21 (δ 1.90) and H-22 (δ 3.51), as well as the lack of significant NOE with the adjacent H-4.

The H-10 olefinic proton exhibited NOESY cross peaks with only those of the nearby H-12, H-13, and H-15, suggesting the *E* configuration for the C-1 double bond. This configuration and that of the adjacent H-11 oxymethine were confirmed by the cross peaks at H-7/H-11 and H-11/H-33 (δ 2.14). Owing to the low reactivity of the allylic 11-hydroxyl group, however, the absolute configurations were not determined by the Mosher method or CD measurement. Consequently, it was determined to be *S* by ozonolysis and comparison of the optical rotation between the degraded product dimethyl malate with those of authentic samples. Thus, the absolute configuration of **1a** was assigned to be 1*E*,3*S*,4*R*,6*S*,11*S*.

The combined 2-D NMR analyses showed that despite noticeable differences in chemical shifts of several proton and carbon signals, **1a** and the minor constituent, **1b**, had the same planar structure. Detailed assignments of the NMR signals revealed that the differences were far more significant in the vicinity of the N-23 foramide than in other portions of the molecule, implying that **1a** and **1b** are indeed rotational isomers of each other (Table 1). This interpretation was supported by the NOESY data, in which the H-34 aldehyde proton at δ 8.02 showed significant cross peaks with the H-24 methylenes at δ 3.29 and 3.21 for **1a**, while the same aldehyde proton at δ 8.11 showed cross peaks with the H-22 methylenes at δ 3.61 and 3.42 for **1b**. Similar phenomena have been reported for tertiary amide-containing compounds such as halichondramides and rhizopodin.[9,10]

Haliclonin A is thought to be biosynthetically originated from 3-alkylpyridine dimers which are widely distributed in

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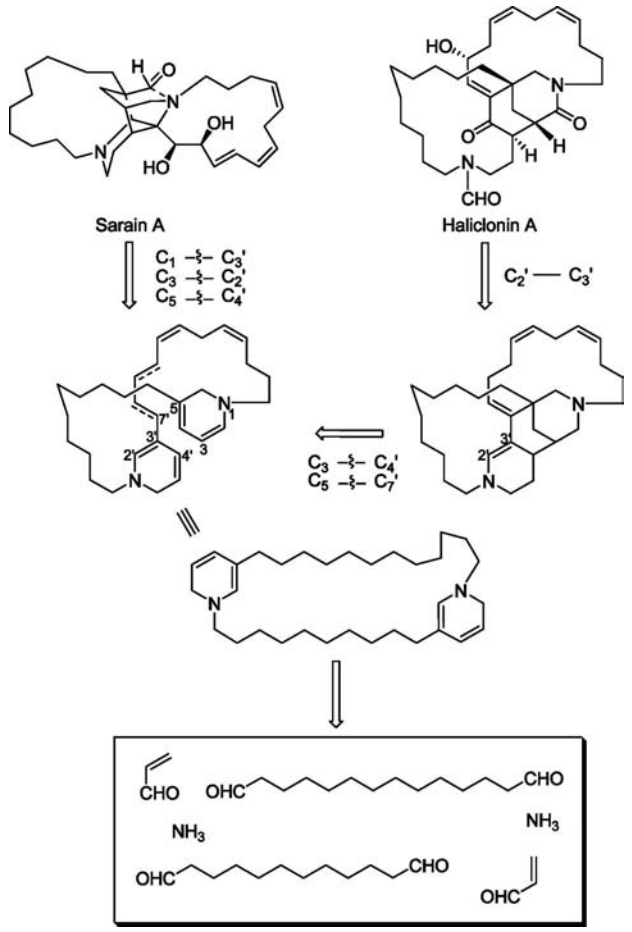
Scheme 1. Possible Retro-Biosynthetic Pathway of Sarain A and Haliclonin A



sponges of the genus *Haliclona* sp. A possible retro-biosynthetic pathway of this compound as well as comparison with that of sarain A[11,12] is illustrated in Scheme 1. A macrocyclic diamine may be first formed from a variety of aldehydes and ammonia. Different modes of cyclization pattern may divaricate sarain A and a haliclonin A precursor which leads to the natural product by oxidative cleavage of a double bond.

In an assessment of bioactivity against Gram-positive and Gram-negative bacteria, compound **1** exhibited moderate antibacterial activity, with minimum inhibitory concentrations of 25, 6.25, 12.5, 12.5, and >100 μg/mL against the test strains *Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* IFO 12708, *Proteus vulgaris* ATCC 3851, and *Escherichia coli* ATCC 25922, respectively. It also displayed moderate cytotoxicity against the K562 leukemia cell line, with an IC_{50} of 15.9 μg/mL (0.03 μmol).

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Supporting Information Available: Full experimental procedures, including spectroscopic and analytical data, along with copies of the ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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