

Cinachyramine, the novel alkaloid possessing a hydrazone and two aminals from *Cinachyrella* sp.

Hiroki Shimogawa, Satomi Kuribayashi, Toshiaki Teruya,
Kiyotake Suenaga and Hideo Kigoshi*

Department of Chemistry, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8571, Japan

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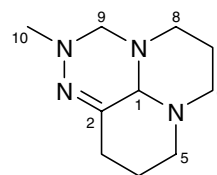
Abstract—The trifluoroacetate salt of cinachyramine (**1**) was isolated from the Okinawan sponge *Cinachyrella* sp. This structure was determined by the spectroscopic analysis and the degradation under acidic conditions. Cinachyramine (**1**) is a novel alkaloid with an unprecedented cage system possessing a hydrazone and two aminals.

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In our continuing search for marine bioactive compounds, we have reported the isolations and structural determinations of spongiacysteine,¹ biselides,² and phormidinines.³ To discover new bioactive compounds, we have investigated the components from Okinawan sponge *Cinachyrella* sp.⁴ Recent studies of this sponge have shown to be rich sources of new marine natural products, such as a cytotoxic macrolide,⁵ unusual steroids,⁶ and aromatic compounds.⁷ In this letter, we report the isolation and structure elucidation of a novel alkaloid from the Okinawan sponge *Cinachyrella* sp.

A sample (0.7 kg) of the marine sponge *Cinachyrella* sp. was collected at Bise in Okinawa, Japan, and extracted with methanol. The methanol extract was partitioned between H₂O and EtOAc, and the H₂O extract was partitioned between BuOH and H₂O. The BuOH layers were concentrated and separated by column chromatography (ODS). Final purification was achieved by reversed-phase HPLC⁸ (ODS) to give the trifluoroacetate salt of cinachyramine (**1**) (1.7 mg) as a colorless oil. Cinachyramine trifluoroacetate⁹ showed weak cytotoxic activity against HeLa S₃ cells with an IC₅₀ of 6.8 μg/mL.

The molecular formula of cinachyramine (**1**) was determined to be C₁₀H₁₈N₄ by HRESIMS (*m/z* 195.1612 [M+H]⁺, Δ +0.2 mmu). The circular dichroism spectrum of cinachyramine trifluoroacetate displayed the Cotton effect (CD [MeOH] λ_{ext} 302 nm [Δε −0.19],



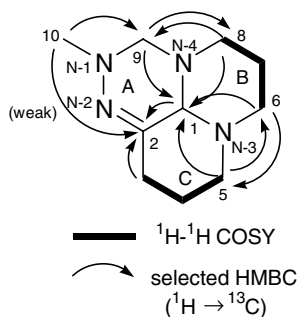
Cinachyramine (**1**)

287 nm [Δε +0.09]). The NMR data for cinachyramine trifluoroacetate are summarized in Table 1. The ¹H, ¹³C NMR, and HMQC spectra indicated the presence of one *N*-methyl, seven sp³-methylenes, and one sp³-methine. Among them, three sp³-methylenes (δ_C 53.2, δ_H 3.41 and 3.18; δ_C 55.1, δ_H 3.56 and 3.19; δ_C 49.0, δ_H 3.09 and 2.80) were ascribed to those bearing a nitrogen atom, and also two carbons (δ_C 68.0, δ_H 3.83 and 3.42; δ_C 77.5, δ_H 4.31) were connected to two nitrogen atoms. A detailed analysis of the ¹H-¹H COSY spectra of cinachyramine trifluoroacetate allowed two partial structures, C3–C5 and C6–C8, to be constructed (Fig. 1). The HMBC correlations H-6a,b/C1 and H-8a,b/C1 suggested the presence of a piperimidine ring (ring B). In addition, the HMBC correlations H-10/C9, H-9a/C8, H-9a,b/C1, H-5b/C1, and H-5b/C6 suggested the connectivity of C10–N1–C9–N4–C1–N3–C5. Furthermore, the degree of unsaturation and one sp²-carbon (δ_C 137.6) of cinachyramine trifluoroacetate revealed the presence of one C=N2 bond and tricyclic structure (rings A, B, and C). The chemical shifts of C3 (δ_C 31.0, δ_H 2.45 and 2.53) and the HMBC correlation H-3a,b/C2 indicated that C3 is connected to C2

* Corresponding author. Tel./fax: +81 29 853 4313; e-mail: kigoshi@chem.tsukuba.ac.jp

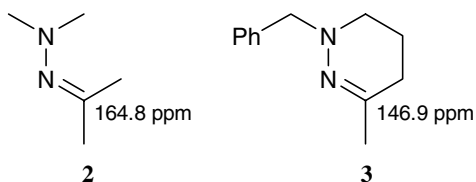
Table 1. NMR spectral data of cinachyramine trifluoroacetate in CD₃OD

No.	¹³ C/ppm ^a	¹ H/ppm ^b mult. (J/Hz)	HMBC ¹ H→ ¹³ C
1	77.5	4.31 (1H, s)	C2, 6, 8
2	137.6		
3a	31.0	2.53 (1H, m)	C2, 5
3b		2.45 (1H, dt, 5.2, 14.4)	C2, 4, 5
4a	23.5 ^c	2.05 (1H, m)	
4b		1.85 (1H, m)	
5a	53.2	3.41 (1H, m)	
5b		3.18 (1H, dt, 2.7, 13.1)	C1, 4, 6
6a	55.1	3.56 (1H, m)	C1, 8
6b		3.19 (1H, dt, 2.6, 12.8)	C1, 5, 7
7a	24.0 ^c	2.10 (1H, m)	
7b		1.88 (1H, m)	
8a	49.0	3.09 (1H, m)	C1, 6, 7, 9
8b		2.80 (1H, dt, 3.0, 12.3)	C1, 6
9a	68.0	3.83 (1H, d, 9.4)	C1, 8
9b		3.42 (1H, d, 9.4)	C1
10	42.7	2.77 (3H, s)	C2, 9

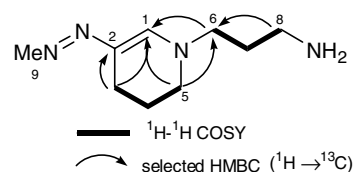
^a Recorded at 100 MHz.^b Recorded at 400 MHz.^c Interchangeable.**Figure 1.** ¹H-¹H COSY and HMBC correlations of cinachyramine trifluoroacetate.

without an N tether. The presence of the C1–C2 bond was suggested by the chemical shifts of C1 (δ_C 77.5, δ_H 4.31) and the HMBC correlation H-1/C2 (ring C).

However, the hydrazone carbon C2 (δ_C 137.6) was observed at much higher field than that of compound **2**¹⁰ (δ_C 164.8). We synthesized the model compound **3**¹¹ with a six-membered ring, and compared the ¹³C chemical shift of the hydrazone carbon (δ_C 146.9) with that of cinachyramine trifluoroacetate, confirming the hydrazone structure in cinachyramine trifluoroacetate. From these results, the structure of cinachyramine trifluoroacetate was determined as shown in structural formula **1**.



Cinachyramine trifluoroacetate was unstable under acidic conditions, and was transformed into **4** on treatment with 0.1 M HCl¹² (Fig. 2). The NMR data for **4**

**Figure 2.** The structure of degradation compound **4**.**Table 2.** NMR spectral data of compound **4** in CD₃OD

C No.	¹³ C/ppm ^a	¹ H/ppm ^b mult. (J/Hz)	HMBC ¹ H→ ¹³ C
1	163.4	8.17 (1H, s)	C3, 5, 6
2	131.5 ^c		
3	19.7	2.40 (2H, t, 6.5)	C1, 2, 4, 5
4	20.5	2.10 (2H, m)	C2, 3
5	49.0 ^c	3.65 (2H, t, 5.7)	C1, 3
6	56.8	3.80 (2H, t, 7.3)	C1, 5, 7, 8
7	26.9	2.10 (2H, m)	C6, 8
8	37.9	3.00 (2H, t, 7.5)	C6, 7
9	40.1	3.42 (3H, s)	

^a Recorded at 100 MHz.^b Recorded at 400 MHz.^c Determined by HMBC experiments.

are summarized in Table 2. The structure of **4** was determined by using the 2D NMR and the mass spectral data (HRESIMS: calcd for C₉H₁₉N₄ [M+H]⁺ *m/z* 183.1610, found 183.1617). The aminal unit at C9 in cinachyramine (**1**) was hydrolyzed with aqueous HCl, and the aminal unit at C1 was cleaved with transformation of a hydrazone to an azo group, providing compound **4**.¹³ The structure of degradation product **4** supported the novel structure of cinachyramine (**1**).

In conclusion, a novel alkaloid, cinachyramine (**1**) was isolated as a trifluoroacetate salt from the Okinawan marine sponge *Cinachyrella* sp. The structure was determined by spectroscopic analysis and degradation under acidic conditions. Cinachyramine trifluoroacetate showed weak cytotoxic activity against HeLa S₃ cells with an IC₅₀ of 6.8 μg/mL.

Acknowledgements

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References and notes

- Kobayashi, K.; Shimogawa, H.; Sakakura, A.; Teruya, T.; Suenaga, K.; Kigoshi, H. *Chem. Lett.* **2004**, *33*, 1262–1263.
- (a) Teruya, T.; Shimogawa, H.; Suenaga, K.; Kigoshi, H. *Chem. Lett.* **2004**, *33*, 1184–1185; (b) Teruya, T.; Suenaga,

- K.; Murayama, S.; Kurotaki, M.; Kigoshi, H. *Tetrahedron* **2005**, *61*, 6561–6567.
3. Teruya, T.; Kobayashi, K.; Suenaga, K.; Kigoshi, H. *Tetrahedron Lett.* **2005**, *46*, 4001–4003.
 4. This sponge was identified by Dr. John N. A. Hooper (Queensland Museum, Australia) and corresponds to Queensland Museum voucher number QM G324173.
 5. Fusetani, N.; Shinoda, K.; Matsunaga, S. *J. Am. Chem. Soc.* **1993**, *115*, 3977–3981.
 6. (a) Songzhi, D.; Xuedong, P.; Dingjun, X.; Houming, W. *Faming Zhuanli Shenqing Gongkai Shuomingshu* **2003**, *7*; (b) Rodriguez, J.; Nunez, L.; Peixinho, S.; Jimenez, C. *Tetrahedron Lett.* **1997**, *38*, 1833–1836.
 7. Liya, L.; Zhiwei, D.; Jun, L.; Hongzheng, F.; Wenhan, L. *Yixueban* **2004**, *36*, 12–17.
 8. Conditions for the isolation of cynachyramine: column, Develosil ODS-HG-5 (20 × 250 mm); solvent, MeOH/H₂O/TFA (10/90/0.1); flow rate, 5.0 mL/min; detection at 254 nm. The retention times of cynachyramine, 22 min.
 9. Cinachyramine trifluoroacetate did not show acute toxicity against mice (LD₉₉ > 100 mg/kg).
 10. Chudek, J. A.; Foster, R.; Young, D. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1285–1289.
 11. *Preparation of 1-benzyl-3-methyl-1,4,5,6-tetrahydropyridazine (3)*. To a solution of 5-chloro-2-pentanone (500 mg, 4.2 mmol) in EtOH (1 mL) was added benzylhydrazine monohydrochloride (660 mg, 4.2 mmol). After being refluxed for 1 h, the mixture was diluted with CHCl₃ (10 mL) and washed with water (3 × 10 mL). The organic layer was dried with Na₂SO₄ and concentrated in vacuum, which was purified by column chromatography on silica gel with CHCl₃/methanol mixtures to give hydrazone **3** (200 mg, 25%) as a yellow oil. ¹H NMR (CDCl₃, 270 MHz) δ_H 7.32–7.20 (m, 5H), 4.10 (s, 2H), 2.52 (t, *J* = 5.4 Hz, 2H), 2.00 (t, *J* = 7.0 Hz, 2H), 1.83 (s, 3H), 1.92–1.79 (m, 2H); ¹³C NMR (CDCl₃, 67.8 MHz) δ_C 146.9 (s) hydrazone carbon, 137.9 (s), 129.0 (d), 128.1 (d), 127.0 (d), 63.2 (t), 46.2 (t), 25.7 (q), 24.1 (t), 20.2 (t); HRMS (ESI) calcd for C₁₂H₁₆N₂+H requires *m/z* 189.1392. Found *m/z* 189.1372.
 12. Cynachyramine trifluoroacetate (1.2 mg) was dissolved in 0.1 M aqueous HCl at room temperature for 5 min. Evaporation followed by reversed-phase HPLC (ODS) with MeOH/H₂O/TFA (10/90/0.1) to give compound **4** (0.2 mg) as a colorless oil.
 13. Configuration at the azo group of compound **4** was not determined.