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Hyrtimomines A–C, New Heteroaromatic Alkaloids from a Sponge *Hyrtios* sp.

Rei Momose,[†] Naonobu Tanaka,[†] Jane Fromont,[‡] and Jun'ichi Kobayashi*[,†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and Western Australian Museum, Locked Bag 49, Weishpool DC, WA 6986, Australia

jkobay@pharm.hokudai.ac.jp

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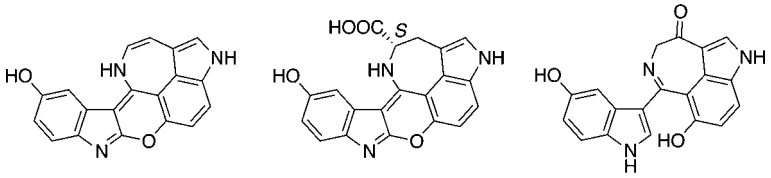
ABSTRACT



hyrtimomine A (**1**) hyrtimomine B (**2**) hyrtimomine C (**3**)

Three new alkaloids, hyrtimomines A–C (1–3), were isolated from an Okinawan marine sponge *Hyrtios* sp. The structures of 1–3 were elucidated on the basis of spectroscopic analysis and application of a phenylglycine methyl ester (PGME) method. Hyrtimomines A (1) and B (2) are heteroaromatic alkaloids possessing a fused hexacyclic 6/5/6/6/7/5 ring system, while hyrtimomine C (3) is an alkaloid consisting of hydroxyindole and azepino-hydroxyindole moieties. Hyrtimomine A (1) exhibited cytotoxicity against KB and L1210 cells.

Marine sponges have been recognized as a rich source of bioactive secondary metabolites with fascinating chemical structures.[1] Among them, sponges belonging to the genus *Hyrtios* are known to be a source of heteroaromatic alkaloids with various structures.[2] Previously, we have reported the isolation of indole alkaloids, hyrtiosins A and B,[3] gesashidine A,[4] and hyrtinadine A[5] from *Hyrtios* spp. We have also isolated alkaloids having a furo[2,3-*b*]pyrazin-2(1*H*)-one moiety, hyrtioseragamines A and B, from *Hyrtios* sp.[6] In our continuing search for structurally unique metabolites from Okinawan marine sponges, we investigated the extracts of *Hyrtios* sp. (SS-163) and isolated three new alkaloids, hyrtimomines A–C (**1**–**3**). In this Letter, we describe the isolation and structure elucidation of **1**–**3**.

The sponge *Hyrtios* sp. (SS-163, 3.3 kg, wet weight) collected off Kerama Islands, Okinawa, was extracted with MeOH, and the extracts were partitioned between EtOAc and water. The EtOAc-soluble materials were partitioned between *n*-hexane and 10% MeOH aq., while the water layer was extracted with *n*-BuOH. Combined 10% MeOH aq.-soluble materials and *n*-BuOH-soluble materials were first fractionated by silica gel column chromatography, followed by fractionation by C_{18} column chromatography. Next, fractions were further purified by MCI gel CHP-20P column chromatography, and final purification was achieved by C_{18} HPLC or HILIC

[†] Hokkaido University.
[‡] Western Australian Museum.

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(7) Hyrtimomine A (**1**): dark-brown amorphous solid; UV (MeOH) λ_{max} 214 (ε 19 610), 293 (9680), 352 (7230 sh), 369 (9810), and 386 (8740) nm; IR (KBr) ν_{max} 3420, 1740–1640 (br), and 1200 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS: *m*/*z* 314.09221 $[M + H]^+$ (calcd for $C_{19}H_{12}N_3O_2$, 314.09240).

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HPLC to afford hyrtimomines A (**1**, 0.00009%, wet weight), B (**2**, 0.00023%), and C (**3**, 0.00012%).

Hyrtimomine A (**1**)[7] was obtained as a dark-brown amorphous solid. The UV spectrum suggested the presence of a conjugated aromatic chromophore. The molecular formula of **1**, $C_{19}H_{11}N_3O_2$, was established by the HRESIMS (*m*/*z* 314.09221 $[M + H]^+$, $\Delta -0.19$ mmu), corresponding to 16 degrees of unsaturation. The ^1H NMR spectrum showed signals of three D_2O-exchangeable, six aromatic, and two olefinic protons, while the ^{13}C NMR spectrum displayed the resonances of 17 aromatic and two olefinic carbons (Table 1). From these data, **1** was elucidated to be a heteroaromatic alkaloid with a highly condensed structure.

The structures of two partial units (units A and B) in **1** were assigned as follows. In unit A (N-1, C-2–C-9, and N-10), the presence of a 3,4-disubstituted-5-hydroxyindole moiety was suggested by analysis of the $^1H-^1H$ COSY and HMBC spectra (Figure 1). $^1H-^1H$ COSY cross-peaks of H-8/H-9 and H-9/10-NH and HMBC correlations for H-8/C-2, H-8/C-3a, and H-9/C-3 disclosed that an ethenamine moiety (C-8 and C-9) was connected to C-3. The geometry of the olefin was assigned as *Z* based on the coupling constant for H-8/H-9 (J = 9.5 Hz). Similarly, the structure of a 2,3-disubstituted-5-hydroxyindole moiety (unit B, N-1′–C-7′a) was deduced. The presence of an oxygen attached to C-2′ was implied by the chemical shift of C-2′ (δ_C 157.0). Thus, the structures of units A and B were assigned as shown in Figure 1.

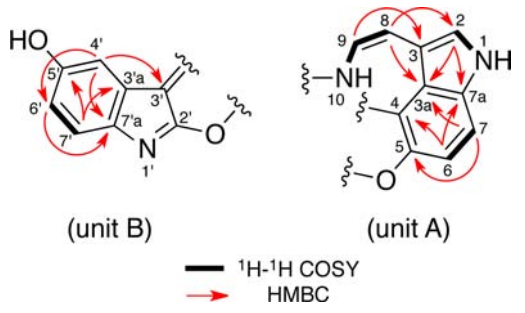

Figure 1. Selected 2D NMR correlations for units A and B in hyrtimomine A (**1**).

In addition to the 2D NMR correlations shown in Figure 1, an HMBC correlation for H-9 to an sp^2 quaternary carbon (δ_C 152.3, C-8′) was observed, suggesting the connectivity of C-9 to C-8′ via N-10. Given the degree of unsaturation of **1** and a ROESY cross-peak of 10-NH/H-4′, the structure of hyrtimomine A (**1**) was elucidated as shown in Figure 2.

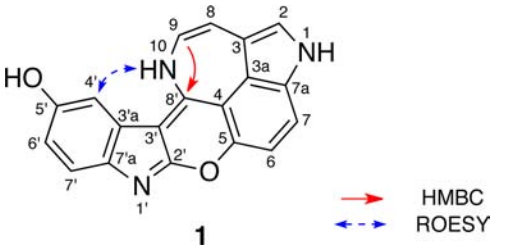

Figure 2. Structure and key 2D NMR correlations of hyrtimomine A (**1**).

Hyrtimomine B (**2**)[8] was obtained as an optically active yellow amorphous solid $\{[\alpha]^{22}_D -276.8$ (*c* 0.027, MeOH)$\}$. The HRESIMS revealed the molecular formula of **2** to be $C_{20}H_{13}N_3O_4$ (*m*/*z* 360.09789 $[M + H]^+$, $\Delta +0.01$ mmu). The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, and the signals due to one nitrogen bearing an sp^3 methine (CH-9), one sp^3 methylene (CH_2-8), and one carboxy group (C-11) in **2** were discerned in place of the resonances of the *Z*-olefine (CH-8 and CH-9) in **1** (Table 1). The connectivity of C-8 to N-10 via C-9 was confirmed by $^1H-^1H$ COSY cross-peaks of H_2-8/H-9 and H-9/10-NH, implying that the carboxy group (C-10) was attached to C-9 (Figure 3). Thus, the gross structure of hyrtimomine B (**2**) was elucidated as shown in Figure 3.

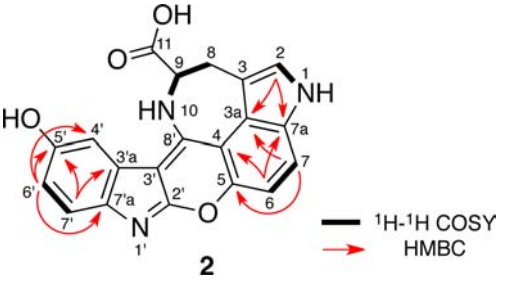

Figure 3. Selected 2D NMR correlations for hyrtimomine B (**2**).

To assign the absolute configuration at C-9, hyrtimomine B (**2**) was converted into the (*S*)- and (*R*)-PGME (PGME = phenylglycine methyl ester) amides (**2a** and **2b**, respectively). The $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) obtained from the ^1H NMR data for **2a** and **2b** indicated the absolute configuration of C-9 in **2** to be *S* (Figure 4).[9]

Hyrtimomine C (**3**)[10] was isolated as a yellow amorphous solid. The HRESIMS indicated the molecular formula of **3** to be $C_{19}H_{13}N_3O_3$ (*m*/*z* 332.10288 $[M + H]^+$, $\Delta -0.11$ mmu).

(8) Hyrtimomine B (**2**): yellow amorphous solid; $[\alpha]^{22}_D$ −276.8 (*c* 0.027, MeOH); UV (MeOH) λ_{max} 219 (ε 11 780), 243 (6480 sh), 290 (5490), 327 (2750 sh), 343 (2980 sh), and 389 (4930) nm; IR (KBr) ν_{max} 3383, 1645, 1592, 1572, and 1364 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS: *m*/*z* 360.09789 $[M + H]^+$ (calcd for $C_{20}H_{14}N_3O_4$, 360.09788).

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(10) Hyrtimomine C (**3**): yellow amorphous solid; UV (MeOH) λ_{max} 215 (ε 24 980), 272 (7430), 295 (7360), 380 (6680), and 474 (3720) nm; IR (KBr) ν_{max} 3427, 2926, 1733–1604 (br), 1588, 1205, and 1138 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRESIMS: *m*/*z* 332.10288 $[M + H]^+$ (calcd for $C_{19}H_{14}N_3O_3$, 332.10297).

Table 1. ^{1}H and ^{13}C NMR Data for Hyrtimomines A–C (**1**–**3**) in DMSO-d_6

	1		**2**		**3**	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	–	11.40 (1H, brs)	–	12.00 (1H, brs)	–	13.15 (1H, brs)
2	125.2	6.97 (1H, brs)	126.3	7.67 (1H, brs)	134.3	8.38 (1H, s)
3	115.0	–	114.2	–	118.0	–
3a	125.7	–	122.6	–	127.1	–
4	108.7	–	106.2	–	107.1	–
5	146.1	–	148.7	–	161.4	–
6	113.1	7.10 (1H, d, J = 8.8 Hz)	110.9	7.57 (1H, d, J = 8.6 Hz)	113.9	7.03 (1H, d, J = 8.6 Hz)
7	119.7	7.43 (1H, d, J = 8.8 Hz)	119.5	7.99 (1H, d, J = 8.6 Hz)	123.7	7.99 (1H, d, J = 8.6 Hz)
7a	134.9	–	132.6	–	130.2	–
8	110.9	5.39 (1H, d, J = 9.5 Hz)	30.4	3.63, 3.48 (1H each, brs)[a]	183.9	–
9	121.9	5.55 (1H, brd, J = 9.5 Hz)	58.7	5.20 (1H, brs)	56.9	4.25 (2H, m)
10	–	8.27 (1H, brs)	–	8.97 (1H, brs)	–	–
11			170.7	13.78 (1H, brs)		
1′	–	–	–	–	–	12.40 (1H, brs)
2′	157.0	–	154.7[b]	–	135.0	8.04 (1H, brs)
3′	95.8	–	94.3	–	110.2	–
3′a	119.7	–	120.4	–	126.5	–
4′	108.1	7.87 (1H, s)	106.2	7.77 (1H, brs)	103.4	6.43 (1H, brs)
5′	154.0	–	153.7	–	153.4	–
6′	114.8	6.94 (1H, d, J = 8.6 Hz)	114.2	6.99 (1H, brd, J = 7.9 Hz)	113.4	6.72 (1H, dd, J = 8.6, 1.9 Hz)
7′	113.5	7.32 (1H, d, J = 8.6 Hz)	113.7	7.45 (1H, d, J = 7.9 Hz)	113.4	7.41 (1H, d, J = 8.6 Hz)
7′a	127.9	–	127.5	–	131.3	–
8′	152.3	–	157.9[b]	–	166.1	–
5-OH						11.18 (1H, brs)
5′-OH		9.67 (1H, brs)		9.66 (1H, brs)		9.12 (1H, brs)

[a] Signals were overlapped with that of HOD. [b] Signals may be interchangeable.

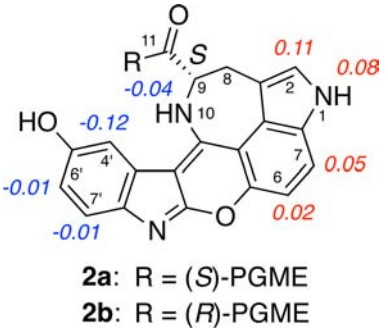


Figure 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the (*S*)- and (*R*)-PGME amides (**2a** and **2b**) of hyrtimoimine B (**2**).

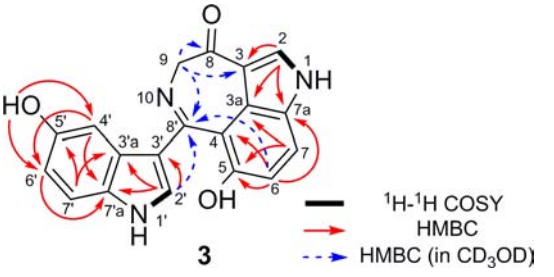
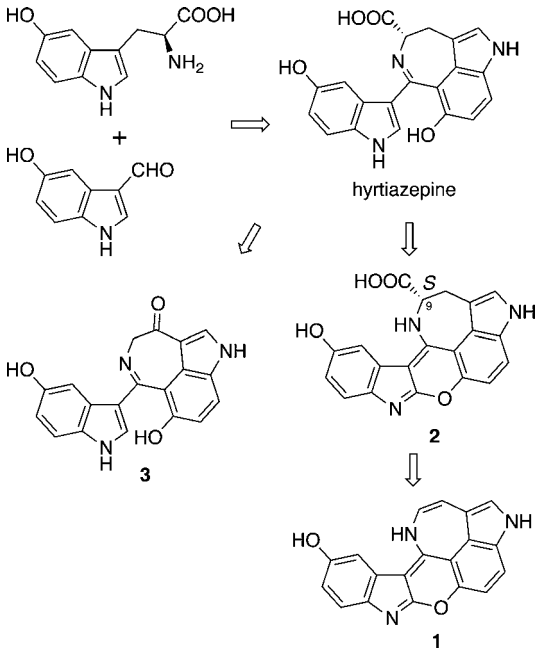


Figure 5. Selected 2D NMR correlations for hyrtimomine C (**3**).

The ^{1}H and ^{13}C NMR spectra revealed the presence of one ketone carbonyl group, one sp^2 quaternary carbon, and one sp^3 methylene adjacent to a nitrogen atom as well as two 5-hydroxyindole moieties (Table 1).

The structures of 3,4-disubstituted-5-hydroxyindole (N-1–C-7a) and 3-monosubstituted-5-hydroxyindole (C-1′–C-7′a) moieties in **3** were confirmed by analysis of the 2D NMR spectra measured in DMSO-d_6, whereas no correlation suggesting the connectivity of CH_2-9 to the other atoms was observed because of its broadening proton signal. On the other hand, the ^{1}H NMR spectrum measured in CD_3OD gave the sharp resonance of H_2-9 {δ_H 4.38 (2H, s)}. In the HMBC spectrum in CD_3OD, correlations for H_2-9 to C-3, C-8, and C-8′ were observed (Figure 5), indicating the connectivities of C-3 to the sp^2 methylene (C-9) through a

Scheme 1. Possible Biogenetic Path of Hyrtimomines A–C (**1**–**3**)



ketone carbonyl group (C-8) and of C-9 to an sp^2 quaternary carbon (C-8′) through a nitrogen atom (N-10). In addition, the connectivities among N-10, C-3′, and C-4 via C-8′ were disclosed by an HMBC cross-peak of H-2′/C-8′ and a ^4J HMBC correlation for H-6/C-8′. Therefore, the structure of hyrtimomine C was elucidated to be **3**.

Hyrtimomines A (**1**) and B (**2**) are structurally unique heteroaromatic alkaloids with a fused hexacyclic 6/5/6/6/7/5 ring system. Hyrtimomine C (**3**) is an alkaloid consisting of a hydroxyindole and azepino-hydroxyindole moieties. These alkaloids have an azepino-indole moiety in common, while some azepino-indole alkaloids, hyrtiazepine,[2c] hyrtioreticulins C and D,[2a] and clavicipitic acid,[11] have been reported to date.

A possible biogenetic path of hyrtimomines A–C (**1**–**3**) from hyrtiazepine is proposed as shown in Scheme 1. Hyrtiazepine seems to be derived from 5-hydroxy-L-tryptophan and 5-hydroxyindole-3-aldehyde.[3] Decarboxylation and oxidation of hyrtiazepine might give hyrtimomine C (**3**). Hyrtimomine B (**2**) might be derived by intramolecular cyclization of hyrtiazepine and followed by decarboxylation to generate hyrtimoimine A (**1**). Although the absolute stereochemistry of hyrtiazepine has not been reported,[2c] the absolute configuration of C-9 in hyrtimomine B (**2**) was coincident with that of 5-hydroxy-L-tryptophan.

Hyrtimomine A (**1**) showed cytotoxicity against human epidermoid carcinoma KB cells (IC_{50} 3.1 μg/mL) and muline leukemia L1210 cells (IC_{50} 4.2 μg/mL) *in vitro*, while hyrtimomines B (**2**) and C (**3**) did not show such cytotoxicity (IC_{50} > 10 μg/mL).

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Supporting Information Available. Experimental section, 1D and 2D NMR spectra of hyrtimomines A–C and the derivatives of hyrtimomine B. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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