

## Hyrtiazepine, an Azepino-indole-Type Alkaloid from the Red Sea Marine Sponge *Hyrtios erectus*<sup>‡</sup>

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Biological and chemical investigations of the methanolic crude extract of the Red Sea marine sponge *Hyrtios erectus* led to the isolation of a novel azepino-indole-type alkaloid named hyrtiazepine (**2**) and 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**), together with the known metabolites hyrtiosulawesine (**1**), 5-hydroxyindole-3-carbaldehyde (**4**), hyrtiosin A (**5**), and hyrtiosin B (**6**). Their structures were elucidated on the basis of mass spectrometry and detailed 2D NMR spectroscopic data. Hyrtiosulawesine (**1**) displayed a significant antiphospholipase A<sub>2</sub> activity with an IC<sub>50</sub> value of 14  $\mu$ M in a fluorometric assay using *Crotalus adamanteus* venom phospholipase A<sub>2</sub>.

Marine sponges of the genus *Hyrtios* (Demospongiae class, Dictyoceratida order, Thorectidae family)<sup>1</sup> continue to be a rich source of structurally diverse metabolites including terpenoids, mainly sesterterpenes,<sup>2–6</sup> and sesquiterpene quinones,<sup>7,8</sup> macrolides,<sup>9,10</sup> and alkaloids.<sup>11–14</sup> Many of them possess important biological activities as illustrated with the promising anticancer althoyrtins (=spongistatins).<sup>15</sup>

In the course of our research program on phospholipase A<sub>2</sub> (PLA<sub>2</sub>) inhibitors from marine sponges, we examined the methanolic extract of the marine sponge *Hyrtios erectus* collected in the Red Sea. The antiphospholipase A<sub>2</sub> bioassay-guided study led to the isolation of the known  $\beta$ -carboline named hyrtiosulawesine (**1**), previously isolated from the Indonesian marine sponge *H. erectus*,<sup>8</sup> as a significant PLA<sub>2</sub> inhibitor. Examination of inactive fractions afforded an unprecedented azepino-indole alkaloid, which we named hyrtiazepine (**2**), and the 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**) together with the known 5-hydroxyindole-3-carbaldehyde (**4**), hyrtiosin A (**5**), and hyrtiosin B (**6**).<sup>14</sup> Recently, chemical investigation of the ethyl acetate of the same sponge afforded three new moderately cytotoxic tryptamine-derived alkaloids, named hyrtioerectines A–C (**7–9**).<sup>11</sup>

This report describes the isolation of compounds **1–6** and structural elucidation of the new compounds **2** and **3**, as well as the antiphospholipase A<sub>2</sub> activity of the known hyrtiosulawesine (**1**).

### Results and Discussion

On the basis of a preliminary anti-PLA<sub>2</sub> assay, the methanolic crude extract of the marine sponge *H. erectus*, which displayed 100% inhibition at a concentration of 1 mg/mL, was selected for its promising anti-inflammatory activity.

A bioassay-guided fractionation on a silica gel column, eluted with CH<sub>2</sub>Cl<sub>2</sub> with increasing amounts of MeOH, furnished one active fraction, which afforded after Sephadex LH-20 chromatography the known hyrtiosulawesine (**1**),<sup>8</sup> as a significant phospholipase A<sub>2</sub> inhibitor (IC<sub>50</sub> = 14  $\mu$ M). In this fluorometric bioassay, manoalide, used as a reference, showed an IC<sub>50</sub> of 0.4  $\mu$ M. To the

best of our knowledge, this is the first time that a  $\beta$ -carboline is reported as a PLA<sub>2</sub> inhibitor.

Inactive fractions were also chemically examined. The CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1) fraction was successively subjected to normal- and reversed-phase HPLC chromatographies to give 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**) and the known 5-hydroxyindole-3-carbaldehyde (**4**). In addition to hyrtiosulawesine (**1**), the CH<sub>2</sub>Cl<sub>2</sub>/MeOH (8:2) fraction also yielded the known metabolites hyrtiosin A (**5**) and hyrtiosin B (**6**).<sup>14</sup> The CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1) fraction furnished, after chromatography on a Sephadex LH-20 and reversed-phase HPLC, the novel azepino-indole-type alkaloid **2**.

Detailed examination of spectroscopic data (UV, IR, 1D and 2D NMR) of the active compound **1** readily established its identity with hyrtiosulawesine, previously isolated from the Indonesian marine sponge *H. erectus*.<sup>8</sup>

Examination of the <sup>1</sup>H and <sup>13</sup>C NMR spectra (see Experimental Section) of inactive compounds **2–6** suggested that they were closely related to each other and they all possess a 5-hydroxyindole moiety. Structures of compounds **4–6** were rapidly identified by comparison of the spectral data with reported values as being 5-hydroxyindole-3-carbaldehyde, hyrtiosin A, and hyrtiosin B, respectively.<sup>14</sup>

Compound **2** was isolated as an optically active, yellow solid, [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –16.9 (*c* 0.13, MeOH). The molecular formula C<sub>20</sub>H<sub>15</sub>N<sub>3</sub>O<sub>4</sub> was deduced by HRFABMS analysis, which exhibited a protonated molecular ion [M + H]<sup>+</sup> at *m/z* 362.1127 ( $\Delta$  –1.3 mmu), indicating the presence of 15 unsaturations in the molecule. The UV absorptions at 227 (4.22), 351 (3.73), and 435 (3.72) nm suggested a conjugated indole moiety, while the MS/MS fragment ion at *m/z* 316 [M – 45]<sup>+</sup> indicated the presence of a carboxylic acid functionality in the molecule. In the <sup>1</sup>H NMR spectrum recorded in CD<sub>3</sub>OD, in addition to signals at  $\delta$  7.85 (s), 7.37 (br s), 6.83 (dd, 8.6, 2.2), and 7.37 (d, 8.6), characteristic of a 3-monosubstituted-5-hydroxyindole nucleus, additional signals at  $\delta$  7.33 (s), 6.85 (d, 8.7), and 7.65 (d, 8.7) were observed, indicating the presence of a 3,4-disubstituted 5-hydroxyindole nucleus. This information was confirmed by HMBC correlations (see Table 1). Moreover, additional signals at  $\delta$  4.34 (dd, 10.0, 1.1), 3.67 (dd, 15.0, 1.1), and 3.10 (dd, 15.0, 10.0) were also observed. Associated HSQC and <sup>1</sup>H–<sup>1</sup>H COSY correlations allowed the establishment of the partial structure –CH<sub>2</sub>–CH–.

HMBC correlations between the methine proton at  $\delta$  4.34 and carbons at  $\delta$  34.7 (C-3) and 114.6 (C-2a) located this partial structure at the C-3 position of the 3,4-disubstituted 5-hydroxyin-

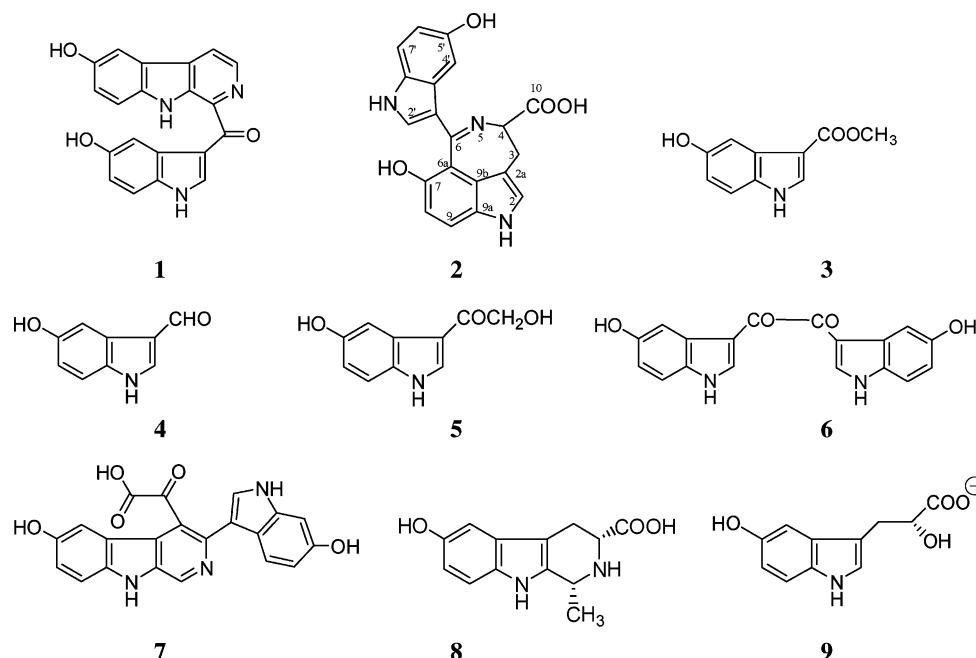
<sup>‡</sup> Dedicated to the memory of Professor Pierre Potier.

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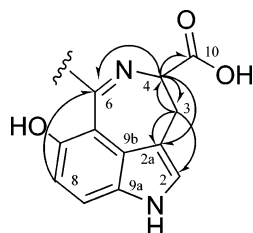


**Figure 1.** Structure of the isolated compounds from the Red Sea marine sponge *H. erectus*.

**Table 1.**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR Data of Hyrtiazepine (**2**) ( $\text{CD}_3\text{OD}$ )

| no. | $\delta_{\text{H}}$ (mult., $J$ in Hz) | $\delta_{\text{C}}$ | HMBC <sup>a</sup>       |
|-----|--|---------------------|-------------------------|
| 2   | 7.33 (s)                               | 127.5               | C-2a, C-9a, C-9b        |
| 2a  |  | 114.6               |                         |
| 3a  | 3.67 (dd, 15.0, 1.1)                   | 34.7                | C-2, C-2a, C-4          |
| 3b  | 3.10 (dd, 15.0, 10.0)                  |                     |                         |
| 4   | 4.34 (dd, 10.0, 1.1)                   | 63.3                | C-2a, C-3, C-6, C-10    |
| 6   |  | 167.1               |                         |
| 6a  |  | 106.6               |                         |
| 7   |  | 158.4               |                         |
| 8   | 6.85 (d, 8.7)                          | 113.8               | C-6, C-6a, C-7, C-9a    |
| 9   | 7.65 (d, 8.7)                          | 122.2               | C-6a, C-7, C-9b         |
| 9a  |  | 132.5               |                         |
| 9b  |  | 127.5               |                         |
| 10  |  | 174.8               |                         |
| 2'  | 7.85 (s)                               | 138.1               | C-3', C-3'a, C-7'a, C-6 |
| 3'  |  | 111.5               |                         |
| 3'a |  | 126.7               |                         |
| 4'  | 7.37 (br s)                            | 104.7               |                         |
| 5'  |  | 155.5               |                         |
| 6'  | 6.83 (dd, 8.6, 2.2)                    | 114.6               | C-4', C-7'a             |
| 7'  | 7.37 (d, 8.6)                          | 114.7               | C-3'a, C-4', C-5'       |
| 7'a |  | 133.1               |                         |

<sup>a</sup> Protons that correlate with carbons



**Figure 2.** Selected key HMBC correlations used to determine the structure of hyrtiazepine (**2**).

dole. Furthermore, significant HMBC correlation between this methine proton at  $\delta$  4.34 and a carbon at  $\delta$  174.8 (C-10) suggested it was vicinal to the carboxylic acid functionality. Furthermore, on the basis of the molecular formula and the degree of unsaturation of **2**, key HMBC correlations of both the aromatic proton at  $\delta$  6.85 (H-8) and the methine proton at  $\delta$  4.34 (H-4) with a carbon at  $\delta$  167.1 (C-6) established the tetrahydroazepine moiety (see Figure 2). Last, HMBC correlation between the proton at  $\delta$  7.85 (s, H-2')

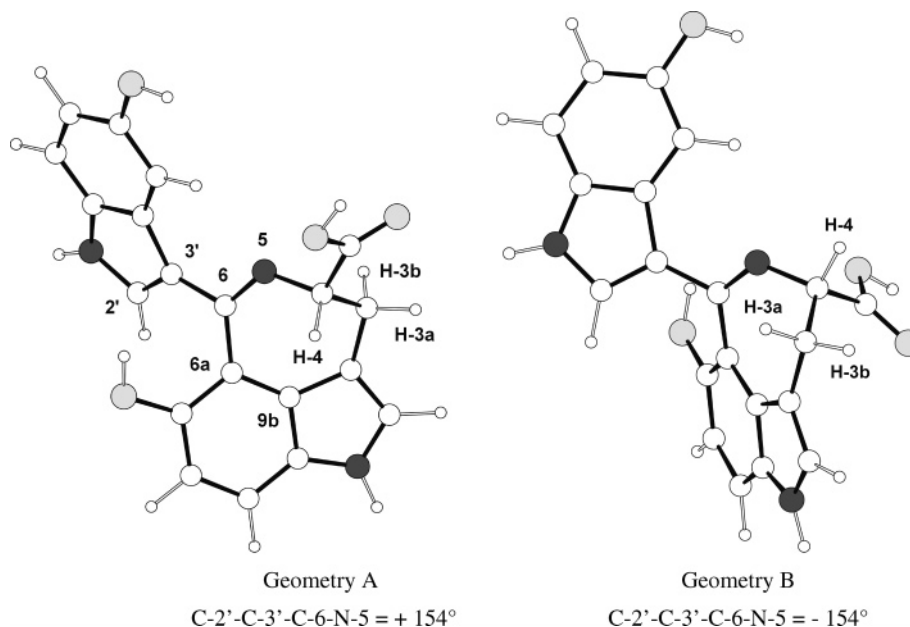
of the remaining indolic nucleus and the carbon at  $\delta$  167.1 (C-6) of the tetrahydroazepine moiety allowed the connection of the two nuclei. Thus, the structure of **2** was determined as being 7-hydroxy-6-(5-hydroxy-1*H*-indol-3-yl)-3,4-dihydro-1*H*-azepino[5,4,3-*cd*]indole-4-carboxylic acid and was named hyrtiazepine.

A conformational analysis of the molecule was carried out by MM2 calculations, which revealed that the molecule of hyrtiazepine (**2**) possessed only two possible geometries (A and B) of the tetrahydroazepine ring with equivalent energies (see Figure 3). The relevant dihedral angles of these two geometries A and B are presented in Table 2.

Moreover, two subconformers exist for each geometry A and B. These correspond to the two possible positions of the indole nucleus around the C-3'–C-6 bond (value of the dihedral angle C-2'–C-3'–C-6–N-5 = ca. +154° or –43° and –154° or +43° for geometry A and B, respectively), the energies of which are comparable. However, the experimentally determined coupling constants  $^3J_{\text{H-4,H-3a}}$  (1.1 Hz) and  $^3J_{\text{H-4,H-3b}}$  (10 Hz) were consistent only with geometry A, possessing values of the dihedral angles H-4–C-4–C-3–H-3a and H-4–C-4–C-3–H-3b of –77° and +163°, respectively.

To the best of our knowledge, hyrtiazepine (**2**) possesses an unprecedented skeleton among marine natural products. The only closely related compound to hyrtiazepine (**2**) is clavicipitic acid (see Figure 4), previously isolated as a mixture of isomers (**10a**, **10b**), from cultures of *Claviceps* strain SD58<sup>16</sup> and *Claviceps fusiformis* 139/2/1G.<sup>17</sup>

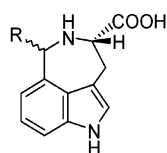
Compound **3** was isolated as an amorphous, pale yellow solid. Its HRCIMS exhibited the protonated molecular ion peak  $[\text{M} + \text{H}]^+$  at  $m/z$  192.0659, consistent with the molecular formula  $\text{C}_{10}\text{H}_{10}\text{NO}_3$  and indicating the presence of 7 unsaturations. In addition to signals of the 5-hydroxy-3-carboxyindole nucleus (see Experimental Section), the  $^1\text{H}$  NMR spectrum recorded in  $\text{CD}_3\text{OD}$  exhibited one methoxyl proton signal at  $\delta$  3.86 ( $\delta_{\text{C}}$  51.2). HMBC correlations between the methoxyl protons with the carbonyl at  $\delta$  168.0 (C-8) furnished the structure of compound **3** as 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester, as depicted in Figure 1. Although compound **3** had been previously prepared synthetically,<sup>18</sup> this is the first report on the isolation of this compound from a natural source, but it cannot be excluded that it may be an artifact due to extraction procedures.



**Figure 3.** Energy-minimized (Macromodel-MM2) representation of one subconformer for geometries A and B of the tetrahydroazepine ring of hyrtiazepine (**2**).

**Table 2.** Dihedral Angles Describing the Geometries A and B of the Tetrahydroazepine Ring of Hyrtiazepine (**2**)

|                      | geometry A | geometry B |
|----------------------|------------|------------|
| $\Delta E$ in kJ/mol | 166.6      | 166.6      |
| C-6-N-5-C-4-C-3      | +72°       | -70°       |
| N-5-C-4-C-3-C-2a     | -80°       | +80°       |
| C-4-C-3-C-2a-C-9b    | +30°       | -30°       |
| C-3-C-2a-C-9b-C-6a   | +10°       | -9°        |
| C-2a-C-9b-C-6a-C-6   | +7°        | -7°        |
| C-9b-C-6a-C-6-N-5    | -37°       | +37°       |
| C-6a-C-6-N-5-C-4     | 0°         | -1°        |
| H-4-C-4-C-3-H-3a     | -77°       | +72°       |
| H-4-C-4-C-3-H-3b     | +163°      | -47°       |



**10a:** R =  $\alpha$ -CH=C(CH<sub>3</sub>)<sub>2</sub>

**10b:** R =  $\beta$ -CH=C(CH<sub>3</sub>)<sub>2</sub>

**Figure 4.** Structure of the clavicipitic acids.

## Experimental Section

**General Experimental Procedures.** UV spectra were obtained in EtOH, using a Kontron-type Uvikon 930 spectrophotometer, and IR spectra were recorded on a Nicolet (Impact 400D) FTIR spectrophotometer.

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with the Bruker XWINNMR program and treated with the Bruker TOPSPIN program in CD<sub>3</sub>OD on a Bruker DRX 400 and/or on a Bruker AVANCE 600 MHz using the signals of the residual solvent protons and carbons as internal references ( $\delta_H$  3.30 and  $\delta_C$  49.0 ppm for CD<sub>3</sub>OD).

The chemical shift values are reported in parts per million, and the coupling constants are in hertz. HMQC and HMBC experiments were acquired using a <sup>1</sup>H-<sup>13</sup>C broadband probehead. The delay preceding the <sup>13</sup>C pulse for the creation of multiple quanta coherences through several bonds in the HMBC was 70 ms.

Mass spectra were recorded on an API Q-STAR PULSAR I from Applied Biosystem and on a JEOL MS 700BE for low- and high-resolution spectra, respectively.

Silica gel column chromatographies were carried out using Kieselgel 60 (230–400 mesh, E. Merck), and gel filtrations were carried out

using LH20 (Sephadex LH20 17-0090-01 Pharmacia Biotech). Fractions were monitored by TLC using aluminum-backed sheets (Si gel 60 F254, 0.25 mm thick) with visualization under UV (254 nm) and Lieberman spray reagent. Analytical reversed-phase HPLC (Kromasil RP18 column K2185, 4.6 × 250 mm, MeOH/H<sub>2</sub>O) was performed with a L-6200A pump (Merck-Hitachi) equipped with an L-4250C UV-vis detector (Merck-Hitachi) and a D-2500 chromato-integrator (Merck-Hitachi).

**Animal Material.** Specimens of *Hyrtios erectus* (Keller, 1891) (order Dictyoceratida, family Thorectidae) were collected by hand using scuba from Safaga at the Egyptian Red Sea coast and identified by Prof. Rob van Soest. A voucher specimen has been deposited at the Zoological Museum of Amsterdam under the registration number 16632 and at the Red Sea Invertebrates collection at the Faculty of Pharmacy, Suez Canal University, as collection number DY-19.

**Extraction and Isolation.** Freshly collected specimens of the marine sponge *H. erectus* (2 kg wet wt) were immediately frozen on site at -20 °C until processed. The sponge was extracted with MeOH, and the methanolic crude extract (20 g) was first fractionated by Si gel CC eluted with CH<sub>2</sub>Cl<sub>2</sub> with increasing amounts of MeOH.

The CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1) fraction (300 mg) was further fractionated on Si gel CC eluted with CH<sub>2</sub>Cl<sub>2</sub> with increasing amounts of acetone to give six subfractions. The 9:1 subfraction (32 mg) was subjected to reversed-phase (MeOH/H<sub>2</sub>O, 1:1 v/v, to 100% MeOH) CC to yield, after further purification by RP HPLC (MeOH/H<sub>2</sub>O, 1:1 v/v, flow rate: 0.8 mL/min,  $t_r$  = 7 min,  $\lambda$  254 nm), 5-hydroxy-1H-indole-3-carboxylic acid methyl ester (**3**) (1 mg). The 7:3 subfraction (17 mg) was subjected to RP HPLC (MeOH/H<sub>2</sub>O, 2:3 v/v, flow rate: 0.8 mL/min,  $t_r$  = 6 min,  $\lambda$  254 nm) to afford the 5-hydroxyindole-3-carbaldehyde (**4**) (1 mg).

The 4:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH fraction (200 mg) yielded, after size exclusion chromatography on Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 1:1 v/v), the known hyrtiosin A (**5**) (1 mg), hyrtiosin B (**6**) (0.7 mg), and hyrtiosulawesine (**1**) (1 mg).

The 1:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH fraction (2.2 g) was chromatographed on Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 1:1 v/v) and purified by RP HPLC (MeOH/H<sub>2</sub>O, 2:3 v/v, flow rate: 0.5 mL/min,  $t_r$  = 11 min,  $\lambda$  423 nm) to afford the novel hyrtiazepine (**2**) (1.2 mg).

**Hyrtiosulawesine (1):** yellow solid; UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 217 (5.16), 305 (4.72), 403 (4.36) nm; IR (NaCl)  $\nu_{max}$  3300, 1640 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  8.89 (1H, s, H-2'), 8.43 (1H, d,  $J$  = 5.1 Hz, H-3), 8.16 (1H,  $J$  = 5.1 Hz, H-4), 8.02 (1H, d,  $J$  = 2.5 Hz, H-4'), 7.57 (1H, d,  $J$  = 2.4 Hz, H-5), 7.53 (d,  $J$  = 8.7 Hz, H-8), 7.33 (1H, d,  $J$  = 8.7 Hz, H-7'), 7.14 (1H, dd,  $J$  = 8.7, 2.4 Hz, H-7), 6.82 (1H, dd,  $J$  = 8.7, 2.5 Hz, H-6'); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75.13 MHz)  $\delta$  154.4 (C, C-5'), 152.6 (C, C-6), 140.2 (C, C-1), 139.1 (CH, C-2'), 137.5 (C, C-9a), 137.4 (C, C-8a), 137.2 (CH, C-3), 132.4 (C, C-4a), 132.3 (C, C-7'a), 129.9 (C, C-3'a), 122.6 (C, C-4b), 119.8 (CH, C-7), 118.5

(CH, C-4), 116.0 (C, C-3'), 113.8 (CH, C-6'), 113.8 (CH, C-8), 113.3 (CH, C-7'), 108.0 (CH, C-4'), 106.7 (CH, C-5); HRCIMS  $m/z$  366.0854  $[M + Na]^+$  (calcd for  $C_{20}H_{13}N_3O_3Na$ , 366.0859).

**Hyrtiazepine (2):** yellow solid;  $[\alpha]_D^{25} -16.9$  (c 0.13,  $CH_3OH$ ); UV (EtOH)  $\lambda_{max}$  (log  $\epsilon$ ) 227 (4.22), 351 (3.73), and 435 (3.72) nm; IR (NaCl)  $\nu_{max}$  3258 (broad), 1625  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR recorded in  $CD_3OD$ , see Table 1; HRFABMS  $m/z$  362.1127  $[M + H]^+$  (calcd for  $C_{20}H_{16}N_3O_4$ , 362.1114).

**5-Hydroxy-1H-indole-3-carboxylic acid methyl ester (3):** yellow needles;  $^1H$  NMR ( $CD_3OD$ , 400 MHz)  $\delta$  7.87 (1H, s, H-2), 7.45 (1H, d,  $J = 2.4$  Hz, H-4), 7.26 (1H, d,  $J = 8.7$  Hz, H-7), 6.75 (1H, dd,  $J = 2.4, 8.7$  Hz, H-6), 3.86 (3H, s, H-9);  $^{13}C$  NMR ( $CD_3OD$ , 75.13 MHz)  $\delta$  168.0 (C, C-8), 153.7 (C, C-5), 133.4 (CH, C-2), 132.7 (C, C-7a), 128.4 (C, C-3a), 113.6 (CH, C-7), 113.4 (CH, C-6), 107.5 (C, C-3), 106.2 (CH, C-4), 51.2 ( $CH_3$ , C-9); HRCIMS  $m/z$  192.0659  $[M + H]^+$  (calcd for  $C_{10}H_{10}NO_3$ , 192.0657).

**5-Hydroxy-indole-3-aldehyde (4):** colorless needles;  $^1H$  NMR ( $CD_3OD$ , 400 MHz)  $\delta$  9.80 (1H, s, H-8), 7.99 (1H, s, H-2), 7.56 (1H, d,  $J = 2.3$  Hz, H-4), 7.29 (1H, d,  $J = 8.7$  Hz, H-7), 6.81 (1H, dd,  $J = 2.3, 8.7$  Hz, H-6);  $^{13}C$  NMR ( $CD_3OD$ , 75.13 MHz)  $\delta$  187.1 (CH, C-8), 154.7 (C, C-5), 139.8 (CH, C-2), 133.3 (C, C-7a), 126.8 (C, C-3a), 119.7 (C, C-3), 114.6 (CH, C-6), 113.6 (CH, C-7), 106.9 (CH, C-4); HRCIMS  $m/z$  162.0553  $[M + H]^+$  (calcd for  $C_9H_8NO_2$ , 162.0551).

**Hyrtiosin A (5):** colorless needles;  $^1H$  NMR ( $CD_3OD$ , 400 MHz)  $\delta$  8.10 (1H, s, H-2), 7.64 (1H, d,  $J = 2.4$  Hz, H-4), 7.27 (1H, d,  $J = 8.8$  Hz, H-7), 6.77 (1H, dd,  $J = 2.4, 8.8$  Hz, H-6), 4.69 (2H, s, H-9);  $^{13}C$  NMR ( $CD_3OD$ , 75.13 MHz)  $\delta$  195.7 (C, C-8), 154.5 (C, C-5), 134.2 (CH, C-2), 132.7 (C, C-7a), 128.1 (C, C-3a), 114.4 (C, C-3), 114.1 (CH, C-6), 113.5 (CH, C-7), 107.2 (CH, C-4), 66.2 ( $CH_2$ , C-9); HRCIMS  $m/z$  192.0664  $[M + H]^+$  (calcd for  $C_{10}H_{10}NO_3$ , 192.0667).

**Hyrtiosin B (6):** colorless needles;  $^1H$  NMR ( $CD_3OD$ , 400 MHz)  $\delta$  8.02 (1H, s, H-2), 7.75 (1H, d,  $J = 2.3$  Hz, H-4), 7.31 (1H, d,  $J = 8.7$  Hz, H-7), 6.82 (1H, dd,  $J = 2.3, 8.7$  Hz, H-6);  $^{13}C$  NMR ( $CD_3OD$ , 75.13 MHz)  $\delta$  191.1 (C, C-8), 155.0 (C, C-5), 138.5 (CH, C-2), 132.9 (C, C-7a), 128.4 (C, C-3a), 114.5 (CH, C-6), 114.2 (C, C-3), 113.7 (CH, C-7), 107.6 (CH, C-4); ESIMS  $m/z$  321.07  $[M + H]^+$ .

**Computational Procedure.** One thousand conformations of hyrtiazepine (2) were generated by the random search Monte Carlo method<sup>22</sup> and optimized by the PRCG minimization method<sup>23</sup> using the MacroModel (version 5.5) program<sup>24</sup> with the MM2 force field.<sup>25</sup> The search was carried out on blocks of 100 Monte Carlo steps until no additional conformation was found to be of lower energy than the current minimum. Duplicated conformations as well as those that had chirality changes were discarded. All the possible conformations within 12.5 kJ/mol (3 kcal/mol) from the global minimum were analyzed.

**Determination of Anti-PLA<sub>2</sub> Activity.** Bioassay-guided fractionation was based on a colorimetric bioassay.<sup>19</sup> Measurements were done with a CERES 900 spectrophotometer. Extracts (1 mg/mL) and fractions (500  $\mu g/mL$ ) were incubated in 96-well culture plates, 1 h at 25 °C, with 4  $\mu g/mL$  PLA<sub>2</sub> from *Crotalus adamanteus* venom (Sigma).

Biological evaluation of the isolated compounds was measured using a fluorometric bioassay.<sup>20</sup> All fluorescence measurements were done with a JY3D spectrofluorometer equipped with a xenon lamp. Hyrtiazepine (2) and manoalide, used as reference, were dissolved in DMSO and incubated with 1  $\mu g/mL$  PLA<sub>2</sub> from *Crotalus adamanteus* venom (Sigma) for 1 h at 25 °C. The maximal concentration of DMSO did not exceed 0.5%.

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**Supporting Information Available:** Photo of the sponge *Hyrtios erectus* taken by P. Sauleau. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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