# Haterumadysins A−D, Sesquiterpenes from the Okinawan Marine Sponge *Dysidea chlorea*

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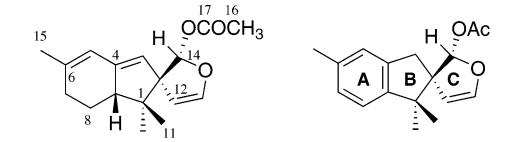
Haterumadysins A−D (**1**−**4**) and two known compounds, spirodysin (**5**) and dehydroherbadysinolide (**6**), were isolated from the sponge *Dysidea chlorea*. Their structures were successfully determined by detailed spectroscopic analysis. Compounds **1**−**5** all showed the ability to inhibit the division of fertilized sea urchin eggs.

Marine sponges of the genus *Dysidea* are a rich source of structurally unique and biologically active compounds. Examples include spiro-sesquiterpenes such as spirodysin and dehydroherbadysinolide, furanosesquiterpenes based on the furodysinin and furodysin skeletons, brominated diphenyl ethers, polychlorinated alkaloids, and other compounds.[1a−e]

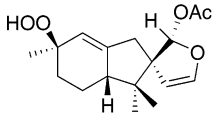
As part of our continuing chemical studies of Okinawan marine organisms, we examined the constituents of the sponge *Dysidea chlorea*, whose crude acetone extract strongly inhibited cell division of fertilized sea urchin eggs. Bioassay-guided fractionation of the extract coupled with $^1$H NMR analyses led to the isolation of four new tricyclic spiro-sesquiterpenes, haterumadysins A−D (**1**−**4**), together with the known compounds spirodysin (**5**) and dehydroherbadysinolide (**6**).[1a,c] Compounds **5** and **6** were first isolated from the Australian sponges *Dysidea herbacea* and *Dysidea* sp., respectively, and have a rare spirolactol or spirolactone framework.




**1**




**2**

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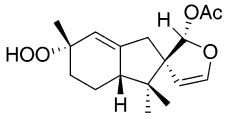


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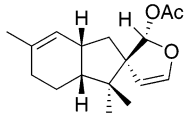
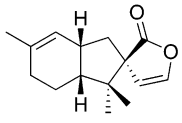



**5**




**6**

The light blue sponge (550 g, wet weight) was collected by hand from the coast of Hateruma Island, Okinawa, and stored at −15 °C before being extracted with acetone. Subsequent partition of the acetone extract between EtOAc/$H_2O$ and repeated purification of the EtOAc extract by silica gel column chromatography followed by ODS HPLC ($H_2O$/MeOH) yielded haterumadysin A (**1**, 0.0010% of wet weight), haterumadysin B (**2**, 0.00047%), haterumadysin C (**3**, 0.00022%), haterumadysin D (**4**, 0.00042%), (−)-spirodysin (**5**, 0.0030%), and (−)-dehydroherbadysinolide (**6**, 0.00042%). (−)-Spirodysin (**5**) and (−)-dehydroherbadysinolide (**6**) were unambiguously identified by comparison of their spectral data with those described in the literature.[1a,c]

Analysis of $^{13}$C NMR (Table 2) and HRESIMS data [*m*/*z* 297.1475 $(M + Na)^+$, Δ +0.8 mmu] for **1** provided a molecular formula of $C_{17}H_{22}O_3$, which accounted for seven degrees of unsaturation. The MS and NMR data (Tables 1 and 2) suggested that **1** was the dehydro analogue of spirodysin (**5**). Detailed analysis of the UV ($\lambda_{max}$ 198 and 244 nm), IR ($\nu_{max}$ 2966, 1745, 1616, 1373, and 1232 $cm^{-1}$), and $^1$H and $^{13}$C NMR data indicated the presence of an ester [$\delta_C$ 170 (s)], a conjugated diene [$\delta_C$ 118.9 (d), 143.5 (s), $\delta_H$ 5.10 (1H, brs) and $\delta_C$ 119.5 (d), 141.9 (s), $\delta_H$ 5.98 (1H, brs)], and a polarized double bond [$\delta_C$ 106.1 (d); $\delta_H$ 5.00 (1H, d, $J$ = 3.0 Hz), 6.43 (1H, d, $J$ = 3.0 Hz)], which was attributed to a 2,3-dihydrofuran moiety. This accounted for five of seven degrees of unsaturations; therefore **1** is tricyclic. The presence of an acetal, a *gem*-dimethyl group, a vinyl methyl, two methylenes, and a methine was also apparent from the NMR data (Tables 1 and 2). The planar structure of **1** was determined by the interpretation of the 2D NMR data (COSY, HMQC, and HMBC, Figure 1). The relative stereochemistry of **1** was established on the basis of NOE data. The NOEs observed between H9/$H_3$10, H9/H14, and $H_3$10/H14 implied that these protons were on the same face of the molecule.

Haterumadysin B (**2**) had a molecular formula of $C_{17}H_{20}O_3$, as determined by HRESIMS [*m*/*z* 295.1298 $(M + Na)^+$, Δ −1.2 mmu], which accounted for an additional degree of unsaturation compared to **1**. The $^1$H NMR spectrum of **2** (Table 1) was similar to that of **1**. The main differences were the presence of three aromatic protons at $\delta_H$ 6.98 (3H, brs), isolated methylene protons at $\delta_H$ 2.83 (1H, d, $J$ = 16.0 Hz) and 3.27 (1H, d, $J$ = 16.0 Hz), and the lack of ethylene protons that resonated at $\delta_H$ 1.35, 1.65, 2.05, and 2.15 in **1**. The planar structure of **2** was established by analysis of the COSY, HMQC, and HMBC data. HMBC correlations of $H_2$3/C1, $H_2$3/C2, $H_2$3/C12, $H_2$3/C4, $H_2$3/C5, $H_2$3/C9, $H_3$15/C5, $H_3$15/C6, and $H_3$15/C7 revealed the presence of a 1,3,6-trisubstituted benzylic moiety (C3−C9). The relative stereochemistry of **2** was established on the basis of NOE data. The NOE observed between $H_3$10 and H14 confirmed that these protons were on the same face of the molecule.

Treatment of **5** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) at 80 °C for 2 h afforded **1** (20%) and **2** (25%).[2] Thus, haterumadysin A (**1**) and haterumadysin B (**2**) were confirmed to be dehydro analogues of spirodysin (**5**).

Analysis of haterumadysin C (**3**) by HRESIMS [*m*/*z* 331.1505 $(M + Na)^+$, Δ −1.6 mmu] provided a molecular formula of

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**Table 1.** $^1$H NMR[a] ($CDCl_3$) Data for Haterumadysins A, B, C, and D (**1**−**4**)

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| position | $\delta$ (mult, *J*/Hz) | $\delta$ (mult, *J*/Hz) | $\delta$ (mult, *J*/Hz) | $\delta$ (mult, *J*/Hz) |
| 3α | 5.10 (brs) | 2.83 (d, 16.0) | 2.36 (ddd, 18.5, 2.0, 2.0) | 2.40 (ddd, 18.5, 1.5, 1.5) |
| 3*β* | | 3.27 (d, 16.0) | 2.80 (brd, 18.5) | 2.79 (brd, 18.5) |
| 5 | 5.98 (brs) | 6.98 (brs) | 5.34 (brd, 2.0) | 5.32 (brs) |
| 7α | 2.05 (dd, 19.3, 5.0) | 6.98 (brs) | 1.79 (ddd, 13.0, 3.0 3.0) | 2.23 (brd, 14.0) |
| 7*β* | 2.15 (m) | | 1.95 (ddd, 13.0, 13.0, 3.0) | 1.35 (ddd, 14.0, 14.0, 4.0) |
| 8α | 1.35 (dddd, 12.5, 12.5, 12.5, 5.0) | 6.98 (brs) | 1.28 (dddd, 13.0, 13.0, 13.0, 3.0) | 1.45 (m) |
| 8*β* | 1.65 (m) | | 1.72 (m) | 1.46 (m) |
| 9 | 2.36 (brd, 12.5) | | 2.18 (m) | 2.00 (m) |
| 10 | 0.74 (s) | 1.13 (s) | 0.65 (s) | 0.66 (s) |
| 11 | 1.00 (s) | 1.17 (s) | 0.90 (s) | 0.91 (s) |
| 12 | 5.00 (d, 3.0) | 4.93 (d, 3.0) | 4.85 (d, 3.0) | 4.85 (d, 3.0) |
| 13 | 6.43 (d, 3.0) | 6.34 (d, 3.0) | 6.39 (d, 3.0) | 6.39 (d, 3.0) |
| 14 | 6.54 (s) | 6.45 (s) | 6.33 (s) | 6.36 (s) |
| 15 | 1.76 (s) | 2.30 (s) | 1.27 (s) | 1.31 (s) |
| $CH_3CO$ | 2.08 (s) | 2.09 (s) | 2.08 (s) | 2.08 (s) |
| OOH | | | 7.32 brs) | 7.20 (brs) |

[a] Recorded at 500 MHz.

**Table 2.** $^{13}$C NMR[a] ($CDCl_3$) Data for Haterumadysins A, B, C, and D (**1**−**4**) and Spirodysin (**5**)

| | $\delta$ | | | | |
|---|---|---|---|---|---|
| C no. | **1** | **2** | **3** | **4** | **5** |
| 1 | 47.4 | 47.7 | 45.6 | 45.7 | 46.9 |
| 2 | 67.6 | 64.9 | 61.3 | 61.2 | 63.1 |
| 3 | 118.9 | 35.8 | 32.7 | 32.8 | 35.9 |
| 4 | 143.5 | 139.7 | 146.9 | 148.6 | 35.3 |
| 5 | 119.5 | 125.0 | 121.7 | 120.4 | 125.0 |
| 6 | 141.9 | 136.2 | 83.6 | 80.1 | 133.0 |
| 7 | 31.1 | 127.7 | 32.2 | 32.0 | 28.5 |
| 8 | 22.1 | 121.8 | 21.9 | 19.3 | 21.3 |
| 9 | 50.4 | 147.8 | 49.6 | 50.2 | 44.5 |
| 10 | 20.6 | 23.5 | 19.3 | 19.3 | 21.9 |
| 11 | 20.3 | 23.8 | 19.6 | 19.6 | 22.9 |
| 12 | 106.1 | 107.8 | 105.8 | 105.9 | 107.1 |
| 13 | 143.8 | 142.9 | 143.5 | 143.4 | 142.5 |
| 14 | 98.2 | 98.9 | 99.1 | 99.0 | 99.3 |
| 15 | 23.9 | 21.3 | 25.0 | 25.0 | 23.9 |
| 16 | 21.1 | 21.1 | 21.2 | 21.2 | 21.1 |
| 17 | 170.0 | 169.9 | 169.9 | 169.9 | 170.1 |

[a] Recorded at 125 MHz.

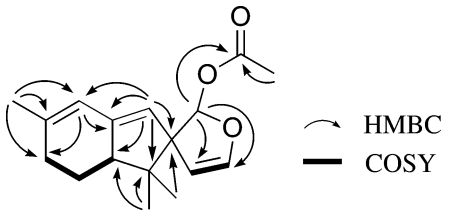


**Figure 1.** Planar structure of haterumadysin A (**1**) based on COSY and selected HMBC correlations.

$C_{17}H_{24}O_5$. The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) resembled those of spirodysin (**5**). The major differences were the appearance of deshielded isolated methylene protons [$\delta_H$ 2.36 (1H, ddd, $J$ = 18.5, 2.0, 2.0 Hz), 2.80 (1H, brd, $J$ = 18.5 Hz)], a deshielded proton [$\delta_H$ 7.32 (1H, brs)], and an oxygenated quaternary carbon at $\delta_C$ 83.6 in **3**. The NMR data and the molecular formula revealed the presence of a trisubstituted double bond at C4 and a hydroperoxy group in compound **3**. A positive iodine-starch test further supported the presence of the hydroperoxy group in **3**. The hydroperoxy group was placed at C6 on the basis of HMBC correlations from H15 to C5, C6, and C7. Detailed analysis of the 2D NMR data led to the planar structure of haterumadysin C (**3**). The relative stereochemistry of **3** was determined by NOEDS experiments (Figure 2). NOE correlations between H9/H7*β*, H9/H8*β*, H9/$H_3$10, and H9/H14 implied that these protons were on the same face of the molecule, whereas NOE correlations between H7α/H8α, H8α/$H_3$11, and H7α/$H_3$15 suggested that these protons were on the opposite face of the molecule.

Haterumadysin D (**4**) had the same molecular formula as that of **3**, $C_{17}H_{24}O_5$, as deduced from HRESIMS [*m*/*z* 331.1510 $(M + Na)^+$, $\Delta$ −1.1 mmu]. The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) were almost identical to those of haterumadysin C (**3**). Extensive analysis of 1D and 2D NMR data and comparison of the NMR data with those of **3** led to the same planar structure as that of **3**. Since the NOEs observed for the portions in the B- and C-rings in **4** resembled those described above for **3**, both compounds had to possess identical stereochemistry at C9, C12, and C14. The methyl group at C6 in **4** was assigned as *β* on the basis of NOEs observed between $H_3$15/H7*β* and $H_3$15/H9; therefore compound **4** was determined to be the C6 epimer of **3**.

Haterumadysins A, B, C, and D (**1**−**4**) completely inhibited the first cleavage of fertilized sea urchin eggs at 1 ppm, and spirodysin (**5**) showed 90% inhibition of the first cleavage of fertilized sea urchin eggs at 1 ppm. However dehydroherbadysinolide (**6**) showed no activity against fertilized sea urchin eggs even at 3 ppm. Accordingly the lactol group in the C-ring seems to be very important for this activity.

Compounds **1**−**4** belong to a small group of sesquiterpenes that contain a furan spirofused to bicyclo[4.3.0]nonane[3] and have a rare spirolactol moiety. In this report, we have described the structures of compounds **1**−**4** and their activity against fertilized sea urchin eggs. We could not conclude that compounds **3** and **4** are natural products, because it is possible that compounds **3** and **4** derive from **5** by oxidation with molecular oxygen in the isolation process. Chemical investigation of the absolute structures of compounds **1**−**4** and biochemical studies to elucidate the structure/activity relationship of spirodysins are in progress.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV and IR spectra were measured using a JASCO V-550 spectrophotometer and a JASCO FT/

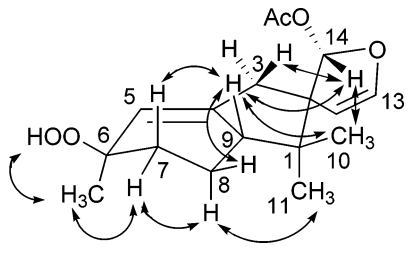


**Figure 2.** Selected NOEs of haterumadysin C (**3**).

IR-300 spectrometer, respectively. The $^1$H, $^{13}$C, and 2D NMR spectra were recorded on a JEOL α-500 spectrometer, and $^1$H and $^{13}$C chemical shifts were referenced to the solvent peaks [$\delta_H$ 7.24 and $\delta_C$ 77.0 in $CDCl_3$]. HRESI mass spectra were determined on a JEOL JMS-LG200 mass spectrometer. Column chromatography was performed on Kieselgel 60 (70−230 mesh, Merck), and HPLC was performed using a COSMOSIL-packed ODS HPLC column (C18, 10 × 250 mm). Analytical TLC was performed using Kieselgel 60 $F_{254}$ DC-fertigplatten (Merck). All solvents used were reagent grade.

**Animal Material.** The light blue sponge was collected by hand from the coast of Hateruma Island, Okinawa, in June 1999 and identified as *Dysidea chlorea* de Laubenfels by Prof. P. R. Bergquist, University of Auckland, New Zealand. The identified sponge was kept frozen until used. A voucher specimen was deposited at the University of the Ryukyus (specimen no. URKU-222).

**Extraction and Isolation.** The sponge *Dysidea chlorea* (550 g, wet weight) was extracted with acetone (1.0 L) twice. After filtration, the extracts were concentrated in vacuo to give an acetone extract (8.3 g). The acetone extract was partitioned between $H_2O$ (200 mL) and EtOAc (300 mL × 2). The EtOAc extract (5.1 g) completely inhibited the first cell division of fertilized sea urchin eggs at 20 ppm. The extract was first chromatographed on Si gel using hexanes with increasing proportions of EtOAc [hexanes (300 mL) → hexanes/EtOAc (5:1, 300 mL → 3:1, 300 mL → 1:1, 300 mL → 3:1, 300 mL) and then EtOAc with increasing proportions of MeOH [EtOAc (300 mL) → EtOAc/MeOH (9:1, 300 mL → 7:1, 300 mL)] to give 13 fractions. An active fifth fraction (890 mg) was subjected to further separation by CC on Si gel using the gradient solvent mixture hexanes/2-propanol (50:1, 50 mL → 25:1, 50 mL → 10:1, 50 mL) and EtOAc (50 mL) to give 14 fractions. Active fractions were combined, and the mixture (87.4 mg) was subjected to ODS HPLC (30% $H_2O$/MeOH; flow rate, 1.5 mL/min) to furnish haterumadysin A (**1**, 5.7 mg), haterumadysin B (**2**, 2.6 mg), haterumadysin C (**3**, 1.2 mg), haterumadysin D (**4**, 2.3 mg), (−)-spirodysin (**5**, 16.3 mg), and (−)-dehydroherbadysinolide (**6**, 2.3 mg).

**Haterumadysin A (1):** colorless oil; $[\alpha]_D^{24}$ −223 (*c* 0.24, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 198 (4.59) and 244 nm (3.98); FT IR $\nu_{max}$ (film) 3010, 2966, 1745, 1616, 1373, and 1232 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* $(M + Na)^+$ 297.1475 (calcd for $C_{17}H_{22}O_3Na$, 297.1467).

**Haterumadysin B (2):** colorless oil; $[\alpha]_D^{24}$ −71 (*c* 0.16, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 200 (4.59) and 275 (3.01); FT IR $\nu_{max}$ (film) 3010, 2965, 1747, 1622, 1373, and 1226 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* $(M + Na)^+$ 295.1298 (calcd for $C_{17}H_{20}O_3Na$, 295.1310).

**Haterumadysin C (3):** colorless oil; $[\alpha]_D^{25}$ −118 (*c* 0.29, $CHCl_3$); FT IR $\nu_{max}$ (film) 3417, 3020, 2965, 1745, 1619, 1371, and 1234 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* $(M + Na)^+$ 331.1505 (calcd for $C_{17}H_{24}O_5Na$, 331.1521).

**Haterumadysin D (4):** colorless oil; $[\alpha]_D^{25}$ −169 (*c* 0.28, $CHCl_3$); FT IR $\nu_{max}$ (film) 3417, 3020, 2967, 1745, 1619, 1377, and 1230 cm$^{-1}$; $^1$H and $^{13}$C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* $(M + Na)^+$ 331.1510 (calcd for $C_{17}H_{24}O_5Na$, 331.1521).

**Conversion of Spirodysin (5) to Haterumadysin A (1) and Haterumadysin B (2).** To a solution of **5** (2.0 mg, 7.2 μmol) in benzene (1.0 mL) was added DDQ (2.0 mg, 23.8 μmol). The mixture was stirred at 80 °C for 2 h and quenched with saturated $NaHCO_3$ solution. The products were extracted with benzene. The organic phase was dried ($Na_2SO_4$) and concentrated in vacuo. The residual oil was purified by reversed-phase HPLC on ODS [COSMOSIL-packed ODS HPLC column (C18, 10 × 250 mm)] using MeOH−$H_2O$ (9:1) to afford **1** (0.4 mg) and **2** (0.5 mg). The $^1$H NMR data and the retention time (12.1 min for **1** and 14.5 min for **2**; flow rate, 2.0 mL/min) of the reaction products **1** and **2** were identical to those of haterumadysin A (**1**) and haterumadysin **B** (**2**), respectively.

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## References and Notes

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