Haterumadienone: A New Puupehenone Congener from an Okinawan Marine Sponge, *Dysidea* sp.

Katsuhiro Ueda,* Tomoyuki Ueta, Eric Richard Oktavianus Siwu,† Masaki Kita,†† and Daisuke Uemura†,†††
Department of Chemistry, Biology and Marine Sciences, University of the Ryukyus, Nishihara-cho, Okinawa 903-0213
†*Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602*
††*Research Center for Materials Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602*
†††*Institute for Advanced Research, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602*

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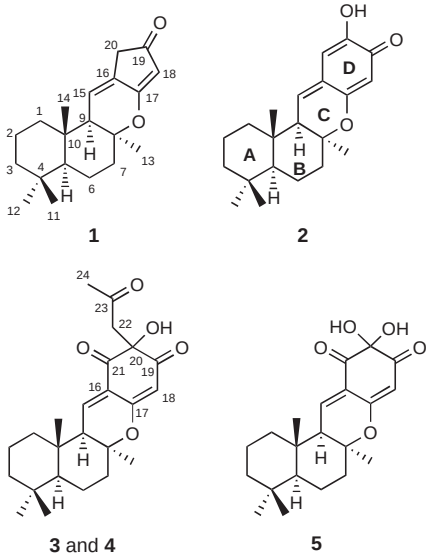
Haterumadienone (**1**), a ring-contracted derivative of puupehenone (**2**), was isolated from a sponge *Dysidea* sp., along with the artificial acetone adducts **3** and **4** of a trione hydrate **5** which could be detected in the sponge. The structures of **1** and the acetone adducts **3** and **4** were successfully determined by detailed spectroscopic analysis. These compounds showed the ability to inhibit the division of fertilized sea urchin eggs.

As part of our continuing search for bioactive metabolites from Okinawan marine organisms, we examined the constituents of the sponge *Dysidea* sp.[1] and isolated three new puupehenone congeners. Puupehenone (**2**)[2] and its derivatives are an important group of marine metabolites because they display a wide range of bioactivities.[3]

The yellowish sponge (750 g) was extracted with acetone. The acetone extract was initially partitioned between EtOAc and water. The cytotoxic EtOAc-soluble material was triturated with hexane. The hexane-soluble part was fractionated by silica-gel column chromatography, followed by ODS HPLC to furnish haterumadienone (**1**, 0.0017% of wet weight)[4] and puupehenone (**2**, 0.05%) as a major constituent. Column chromatography (silica gel) of the hexane-insoluble part and subsequent ODS column chromatography yielded acetone adducts **3** and **4** as a mixture [**3**/**4** (10:9)]. This mixture was then purified by repeated ODS HPLC with 30% H_2O/MeOH to give more polar **3**[5] and **4** (Scheme 1).[6] Puupehenone (**2**) was unambiguously identified by comparison of its spectral data with values in the literature.[2,3b]



Scheme 1.

Analysis of **1** by ^{13}C NMR (Table 1) and HRESIMS [*m*/*z* $(M + Na)^+$ 323.2017, calcd for $C_{20}H_{28}O_2Na$, 323.1988] provided a molecular formula. Although the ^{1}H NMR signals in the aliphatic region were almost identical to those of puupehenone (**2**), **1** showed one less carbon than **2**. Detailed analysis of the IR (ν_{max} 2920, 1696, 1573, 1405, 1175 cm^{-1}), ^{1}H and ^{13}C NMR data (Table 1) indicated the presence of a conjugated ketone (C19), two trisubstituted double bonds (C15–C16 and C17–C18), four methyls and an isolated methylene (C20) in the five-membered ring ($J = 20$ Hz).[7] The major spin systems (**a**, **b**, and **c**) were revealed to be as shown in Figure 1 based on ^{1}H–^{1}H COSY and HMBC data. The partial structures (**a**, **b**, and **c**) and other fragments (C11–C4–C12, C10–C14, C13–C8 and C16–C17) were connected using HMBC correlations (Figure 1) to give the planar structure of the **A**-, **B**-, and **C**-ring moiety. At this point in the structure determination, three fragments (an isolated methylene, a ketone and a sp^2 methine) were identified, but not assembled. Therefore, the one remaining degree of unsaturation corresponds to the presence of a cyclopentenone ring as described before, which was also confirmed by an HMBC experiment (Figure 1). The relative stereochemistry of **1** was established based on NOEDS data. The NOEs observed between Me14/H2b, Me14/Me11, M14/H15, H1a/H5, and Me13/H9 revealed a *trans-anti-cis* fusion of **A**/**B**/**C** rings, which was the same as in puupehenone (**2**).

The molecular formula of **3**, $C_{24}H_{32}O_5$, was established by HRESIMS [*m*/*z* $(M + Na)^+$ 423.2154, calcd for $C_{24}H_{32}O_5Na$, 423.2149] and the ^{13}C NMR spectrum. The ^{1}H NMR spectrum was similar to that of puupehenone (**2**). The main differences

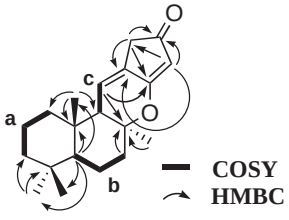


Figure 1. Planar structure of haterumadienone (**1**) based on 2D NMR data.

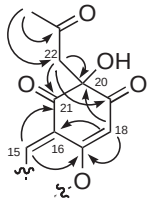


Figure 2. Key HMBC correlations in the acetone adduct **3**.

Table 1. NMR data for **1** and **3**

	1[a]		**3**[b]	
No.	^{13}C	^{1}H (Hz)	^{13}C	^{1}H (Hz)
1	39.4 t	0.67 dt (5.5, 12.5)	40.1 t	1.23 m
		1.26 m		1.77 br d (12.0)
2	18.3 t	1.25 m	18.1 t	1.45 m
3	41.8 t	1.02 dt (4.0, 13,5)	41.4 t	1.19 m, 1.42 m
		1.31 m		
4	33.1 s		33.4 s	
5	53.7 d	0.56 dd (2.0, 11.5)	54.0 d	1.01 dd (2.0, 11.5)
6	18.4 t	1.25 m, 1.36 m	18.3 t	1.50 m, 1.57 m
7	39.4 t	1.15 dt (5.0, 14.0)	38.8 t	1.59 m, 2.23 m
		1.96 td (3.0, 14.0)		
8	80.1 s		79.3 s	
9	53.8 d	1.31 d (6.5)	55.0 d	2.20 d (6.5)
10	39.8 s		41.3 s	
11	22.0 q	0.74 s	21.9 q	0.82 s
12	33.6 q	0.81 s	33.7 q	0.90 s
13	28.5 q	0.94 s	28.8 q	1.26 s
14	14.5 q	0.68 s	15.3 q	0.76 s
15	121.2 d	5.24 d (6.5)	144.8 d	7.46 dd (6.5, 1.5)
16	132.9 s		128.5 s	
17	177.9 s		166.6 s	
18	110.1 d	5.65 s	104.7 d	5.68 d (1.5)
19	199.2 s		193.6 s	
20	37.8 t	2.78 d (20.0)	80.9 s	
		2.82 d (20.0)		
21			191.4 s	
22			52.4 t	2.97 d (14.5)
				3.60 d (14.5)
23			205.5 s	
24			31.6 q	2.18 s
OH				3.90 br s

[a]500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR in C_6D_6. [b]500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR in $CDCl_3$.

were the presence of a singlet methyl signal at δ 2.18, isolated methylene protons at δ 2.97 and 3.60, and the lack of an olefinic proton. Interpretation of the 1D and 2D NMR spectra indicated that **3** had the same **A**-, **B**-, and **C**-ring moiety as **1** or **2**. In the remaining part of **3**, the presence of an acetonyl group, two ketones, an oxygenated quarternary carbon (C20) and a hydroxy group were revealed based on ^{1}H, ^{13}C, and HMBC NMR data. The HMBC correlations (Figure 2) were used to connect these partial structures, establishing the structure of ring **D** (Figure 2). Since the NOEs observed for the **A**, **B**, and **C**-ring moiety in **3** resembled those described above for **1**, both compounds had to possess an identical stereochemistry for the **A**-, **B**-, and **C**-ring moiety.

The NMR spectra of compound **4**[6] are almost identical to those of **3**. The structure of **4** was spectroscopically determined by 2D NMR experiments and by comparison of its NMR data with those of **3**.

An interesting ring-contraction biogenetic pathway from puupuhenone (**2**) to haterumadienone (**1**) might proceed via benzylic acid rearrangement of the diketone form of **2**, followed by oxidative decarbonylation. Haterumadienone (**1**) is the first ring-contracted congener which possesses ring **C**[8] of **2**.

Compounds **3** and **4**, which might be formed by aldol condensation between the trione hydrate **5** and acetone in the extraction process, were epimers at C20 of each other. The assignment of the stereochemistry at C20 of **3** and **4** is in progress. These acetone adducts are the most reasonable adducts because they have less electrostatic interactions between the ketonic groups than other adduct structures (1,2-diketones). The trione hydrate **5** could be detected in the sponge extract with ethyl acetate instead of acetone.[9]

Haterumadienone (**1**) completely inhibited the cell division of fertilized sea urchin eggs at concentration of 2.5 μg/mL. Puupehenone (**2**), and compounds **3** and **4** arrested the division of fertilized sea urchin eggs by 100% at concentration of 1 μg/mL.

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References and Notes

1 The taxonomical assignment was performed by Prof. P. R. Bergquist, University of Auckland, New Zealand.
2 B. N. Rabi, H. P. Perzanowski, R. A. Ross, T. R. Erdman, and P. J. Scheuer, *Pure Appl. Chem.*, **51**, 1893 (1979).
3 a) P. Amade, L. Chevelot, H. P. Perzanowski, and P. J. Scheuer, *Helv. Chim. Acta*, **66**, 1672 (1983). b) M. T. Hamann and P. J. Scheuer, *J. Org. Chem.*, **58**, 6565 (1993). c) S. S. Nasu, B. K. S. Yeung, M. T. Hamann, P. J. Scheuer, M. Kelly-Borges, and K. Coins, *J. Org. Chem.*, **60**, 7290 (1995). d) M.-L. Bourguet-Kondracki, C. Debitus, and M. Guyot, *Tetrahedron Lett.*, **37**, 3861 (1996). e) I. C. Pina, M. L. Sanders, and P. Crews, *J. Nat. Prod.*, **66**, 2 (2003).
4 Colorless oil (13 mg); $[\alpha]_D^{25}$ −57° (*c* 0.14, $CHCl_3$); FDMS *m*/*z* 400 (M^+); ^{1}H and ^{13}C NMR data are given in Table 1.
5 Colorless oil (2.5 mg, polar); $[\alpha]_D^{25}$ −76° (*c* 0.12, $CHCl_3$); FT/IR (film) ν_{max} 3400, 1730, 1260, 1240 cm^{-1}; UV λ_{max} 215 (ε 42000), 277 (ε 15000) nm; ^{1}H and ^{13}C NMR data are given in Table 1.
6 Colorless oil (3 mg, less polar); $[\alpha]_D^{25}$ +183° (*c* 0.16, $CHCl_3$); HRESIMS *m*/*z* $(M + Na)^+$ 423.2207, calcd for $C_{24}H_{32}O_5Na$, 423.2149; FT/IR (film) ν_{max} 3400, 1730, 1260, 1240 cm^{-1}; UV λ_{max} 215 (ε 42000), 277 (ε 15000) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.83 (3H, s), 0.88 (3H, s), 0.91 (3H, s), 1.01 (1H, m), 1.15 (3H, s), 1.19 (1H, m), 1.23 (1H, m), 1.42 (1H, m), 1.45 (2H, m), 1.50 (1H, m), 1.57 (1H, m), 1.60 (1H, m), 1.76 (1H, m), 2.18 (1H, d, J = 7.0 Hz), 2.23 (3H, s), 2.24 (1H, m), 2.86 (1H, d, J = 14.5 Hz), 2.99 (1H, d, J = 14.5 Hz), 5.68 (1H, d, J = 1.5 Hz), 7.33 (1H, dd, J = 6.5, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.4 (q), 18.1 (t), 18.2 (t), 21.9 (q), 28.5 (q), 32.1 (q), 33.3 (s), 33.7 (q), 38.9 (t), 40.2 (t), 41.2 (s), 41.4 (t), 52.4 (t), 53.9 (d), 54.6 (d), 79.3 (s), 82.8 (s), 104.4 (d), 129.5 (s), 144.5 (d), 165.9 (s), 192.5 (s), 193.0 (s), 205.1 (s).
7 L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London (1969), p 271.
8 Molokinenone,[3c] another ring-contracted congener, is a diol that results from the cleavage of an ether bond in ring **C**.
9 Formation of the trione was observed by TLC and ESIMS upon treatment of the acetone adducts **3** and **4** with NaOH in aqueous MeOH. Although we tried to extract the sponge with EtOAc instead of acetone, we did not observe the presence of the trione itself. However, a more polar fraction, which was eluted with EtOAc–MeOH (9.5:0.5) in column chromatography, was treated with acetone to furnish the acetone adducts **3** and **4**. Therefore, the trione hydrate **5** should be the major form in the equilibrium in the sponge extract.

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