

HETEROCYCLES, Vol. 72, 2007, pp. 655 - 663. © The Japan Institute of Heterocyclic Chemistry
Received, 14th November, 2006, Accepted, 21st December, 2006, Published online, 22nd December, 2006. COM-06-S(K)17

CYTOTOXIC HATERUMADIENONE CONGENERS FROM THE OKINAWAN MARINE SPONGE *DYSIDEA* SP.

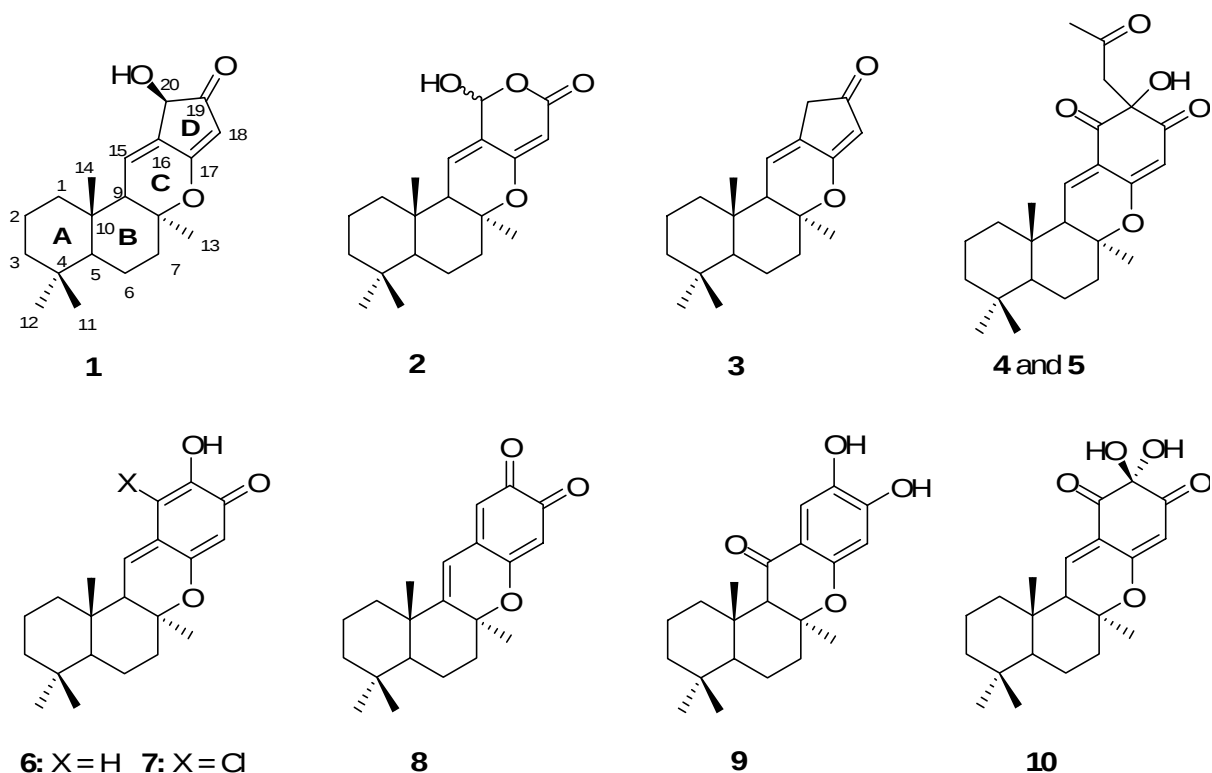
Katsuhiko Ueda,^{a,*} Takayuki Ogi,^a Atsushi Sato,^a Eric R. O. Siwu,^b Masaki Kita,^c
and Daisuke Uemura^{b,d}

^a Department of Chemistry, Biology and Marine Sciences, University of the Ryukyus, Nishihara-cho, Okinawa 903-0213, Japan; ^b Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602, Japan; ^c Research Center for Materials Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602, Japan; ^d Institute for Advanced Research, Nagoya University, Furo-cho, Chikusa, Nagoya 46-8602, Japan, E-mail: kueda@sci.u-ryukyu.ac.jp

Abstract - 20-Hydroxyhaterumadienone (**1**) and haterumadienelactol (**2**), new haterumadienone (**3**) congeners, were isolated from the sponge *Dysidea* sp. together with puupehenone (**6**) and three known metabolites (**7-9**) related to **6**. The absolute stereochemistry of **1** and the relative stereochemistry of **2** were determined by spectroscopic and chemical analyses. The metabolites (**1** and **2**) inhibited the division of fertilized sea urchin eggs.

INTRODUCTION

Marine sponges are a rich source of structurally unique and biologically active terpenoids. Puupehenone (**6**), which was first isolated in 1979 from a Hawaiian sponge,¹ and its derivatives are an important group of marine metabolites because they display a wide range of properties including cytotoxic, antiviral, antitumor, antifungal, antimalarial, antituberculosis and immunomodulatory activities.¹⁻¹⁰ As part of our continuing chemical studies on Okinawan marine organisms, we examined the constituents of the sponge *Dysidea* sp., whose extract strongly inhibited the cell division of fertilized sea urchin eggs. In our recent report, we described the isolation and structure elucidation of haterumadienone (**3**) and the artificial acetone adducts (**4** and **5**) from the toxic extract of the sponge.¹⁰ Further bioassay- and ¹H NMR-guided fractionation of the same extract led to the isolation of 20-hydroxyhaterumadienone (**1**),



haterumadienelactol (**2**) along with the known (+)-puupehenone (**6**),^{1,3,8,12} chloropuupehenone (**7**),³ puupehedione (**8**),³ and 15-oxopuupehenol (**9**).⁴ The relative structures of the **A**-, **B**- and **C**-ring moieties in **1** and **2** were established by NOE experiments and coupling constant analysis. The relative stereochemistry of **1** at C20 was deduced from a series of NOEDS spectral analyses of the (*S*)- and (*R*)-MTPA esters and the absolute stereochemistry of C20 was determined using the modified Mosher's method.¹¹ The absolute stereochemistry of the **A**-, **B**- and **C**-ring moieties of **2** was tentatively deduced to be as depicted in **2** based on an assumption regarding the biosynthetic relationship between **1**-**9**. The known compounds were identified by comparison of their spectral data with values in the literature. Puupehenone (**6**) was the major constituent of the sponge. In this report, we have described the isolation, structure elucidation and biological activity of **1** and **2**, along with detailed experimental procedures for **3**, **4** and **5**.

RESULTS AND DISCUSSION

The yellow encrusting sponge (750 g) was collected by hand from the coast of Hateruma Island, Okinawa, and stored at -15°C before being extracted with acetone. The acetone extract was initially partitioned between ethyl acetate and water. The cytotoxic EtOAc-soluble material was triturated with hexanes and filtered. The hexane-insoluble part was fractionated by silica-gel column chromatography with a gradient from hexanes to EtOAc to MeOH, followed by ODS HPLC (MeOH) to furnish 20-hydroxyhaterumadienone (**1**, 0.0008% of wet weight), haterumadienelactol (**2**, 0.00066%) and acetone

Table 1. NMR data for **1**, **2** and **3**.

1^a			2^a		3^b	
C no.	¹³ C	¹ H (Hz)	¹³ C	¹ H (Hz)	¹³ C	¹ H (Hz)
1	39.5	1.10 dt (4.0, 12.5) 1.72 brd (12.5)	39.8, 39.7 ^c	1.14 m, 1.63 m	39.4	0.67 dt (5.5, 12.5) 1.26 m
2	18.0	1.40 m	18.0	1.40 m	18.3	1.25 m
3	41.6	1.16 dt (4.0, 12.5) 1.42 m	41.6, 41.5 ^c	1.15 m, 1.39 m	41.8	1.02 dt (4.0, 13.5) 1.31 m
4	33.1		33.1		33.1	
5	54.1	0.94 dd (2.5, 12.5)	53.8	0.92 m	53.7	0.56 dd (2.0, 11.5)
6	18.3	1.54 m	18.2	1.55 m	18.4	1.25 m, 1.36 m
7	39.2	1.65 dt (5.0, 14.5) 2.22 td (3.0, 14.5)	38.8, 38.9 ^c	1.55 m, 2.16 m	39.4	1.15 dt (5.0, 14.0) 1.96 td (3.0, 14.0)
8	81.6		78.4, 78.5 ^c		80.1	
9	53.6	1.98 d (6.5)	54.8, 54.6 ^c	1.96 d (7.0) 1.98 d (7.0) ^c	53.8	1.31 d (6.5)
10	40.1		40.1, 40.0 ^c		39.8	
11	21.9	0.82 s	21.8	0.83 s	22.0	0.74 s
12	33.6	0.89 s	33.6, 33.1 ^c	0.89 s	33.6	0.81 s
13	28.9	1.26 s	28.3, 28.2 ^c	1.24 s, 1.18 s ^c	28.5	0.94 s
14	14.3	0.78 s	14.6, 14.5 ^c	0.78 s, 0.81 s ^c	14.5	0.68 s
15	123.3	6.26 d (6.5)	133.3, 132.6 ^c	6.41 d (6.0) 6.55 d (6.0) ^c	121.2	5.24 d (6.5)
16	135.1		128.2		132.9	
17	177.8		161.5, 162.4 ^c		177.9	
18	105.0	5.34 brs	93.8, 94.3 ^c	5.28 s, 5.25 s ^c	110.1	5.65 s
19	202.1		166.6		199.2	
20	70.8	4.60 s	94.1, 93.6 ^c	6.05 s, 6.15 s ^c	37.8	2.78 d (20.0) 2.82 d (20.0)

^a Recorded at 500MHz ¹H NMR and 125 MHz ¹³CNMR in CDCl₃. ^b Recorded at 500MHz ¹H NMR and 125 MHz ¹³C NMR in C₆D₆.^c Values of a minor compound at equilibrium.

adducts (**4** and **5**) as a mixture [**4/5** (10:9)] together with the known puupehedione (**8**, 0.00067%) and 15-oxopuupehenol (**9**, 0.0004%). This mixture was then purified by repeated ODS HPLC with 30% H₂O/MeOH to give pure **4** (0.00033%) and **5** (0.0004%). Silica gel chromatography (hexanes/EtOAc gradient) of the hexane-soluble part and subsequent ODS chromatography (10% H₂O/MeOH) yielded haterumadienone (**3**, 0.0019%), puupehenone (**6**, 0.05%) and chloropuupehenone (**7**, 0.00067%).

Analysis of **1** by ¹³C NMR (Table 1) and HRESIMS [*m/z* 339.1947(M+Na)⁺, calcd for C₂₀H₂₈O₃ Na, 339.1936] gave a formula of C₂₀H₂₈O₃, which accounted for seven degrees of unsaturation. The ¹H NMR signals in the aliphatic region were almost identical to those of puupehenone (**6**). Compound (**1**) showed one less carbon than **6**. Detailed analysis of the UV [$\lambda_{\max}$ 284 (ϵ 20000) nm], IR ($\nu_{\max}$ 3314, 2926, 1660, 1594, 1419 and 1164 cm⁻¹) and NMR data (Table 1) indicated the presence of a conjugated ketone (δ_{C} 202.1), a conjugated diene [δ_{C} 123.3 (d), 135.1 (s), δ_{H} 6.26 (d, *J*=6.5 Hz) and δ_{C} 177.8 (s), 105.0 (d), δ_{H} 5.34 (d, brs)], an oxygenated quarternary sp³ carbon (δ_{C} 81.6), an isolated oxygenated methine (δ_{C} 70.8, δ_{H} 4.60) and four singlet methyls (δ_{C} 21.9, 33.6, 28.9, 14.3 ; δ_{H} 0.82, 0.89, 1.26, 0.78). Of the four methyls, two were part of a *gem*-dimethyl group as indicated by HMBC correlations of H₃11/C12,

H₃12/C11, H₃11/C4 and H₃12/C4, one could be attached to the oxygenated quaternary sp³ carbon at δ_C 80.1 based on an HMBC correlation between H₃13 and C8, and another was located at a bridge-head position based on an HMBC correlation of H₃14 to C10. The major spin systems (**a**, **b** and **c**) were revealed to be as shown in Figure 1 based on ¹H-¹H COSY and HMBC data. The partial structures (**a**, **b** and **c**) and other fragments (C11-C4-C12, C10-C14, C13-C8 and C16-C18) were connected using HMBC correlations to give the planar structure of the **A**-, **B**- and **C**-ring moieties (Figure 1). Since this and the presence of a ketone accounted for six of the seven degrees of unsaturation, **1** is tetracyclic. At this point in the structure determination, three fragments (an oxygenated methine, a ketone and an sp² methine) were identified, but not assembled. Therefore, the one remaining degree of unsaturation corresponds to the presence of a cyclopentenone ring, which was confirmed by HMBC correlations of H18/C16, H18/C19, H18/C20, H20/C15, H20/C16, H20/C17 and H20/C19, with a hydroxyl group clearly positioned at C-20 (Figure 1). The relative stereochemistry of **1** was established based on NOE data. The NOEs observed between Me14/Me11, Me14/H15, and Me13 /H9 revealed a *trans-anti-cis* fusion of **A/B/C** rings, which was the same as in puupehenone (**6**) (Figure 2). The relative stereochemistry of **1** at

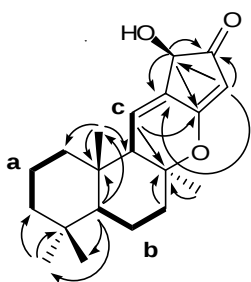


Figure 1. Partial structures (**a**, **b** and **c**) of 20-hydroxyhaterumadienone (**1**) based on COSY (bold line) and some important HMBC correlations (arrows).

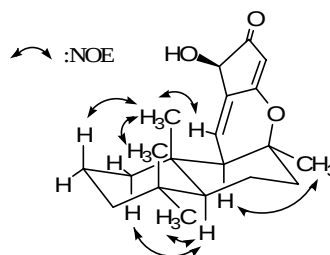


Figure 2. Selected NOE correlations of 20-hydroxyhaterumadienone (**1**).

C20 was deduced from a series of difference NOE experiments of the (*S*)- and (*R*)-MTPA esters, and the absolute stereochemistry of C20 was determined using the modified Mosher's method.¹¹ Esterification of **1** gave the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**). The enhancement of OMe protons in **1a** (or aromatic protons in **1b**) upon irradiation of the Me14 protons of **1a** (or **1b**) allowed the ester group to be assigned as β because the conformers shown in Figure 3 are accepted as preferable for MTPA esters (the carbonyl proton at C20, the C=O oxygen and the CF₃ group are eclipsed to each other).¹² The ¹H NMR signals of the MTPA esters were assigned based on the 2D NMR data, and the $\Delta\delta$ values ($\delta_S - \delta_R$, ppm) were then calculated as shown in Figure 4. Thus, the absolute stereochemistry of 20-hydroxyhaterumadienone (**1**) was determined to be 5*S*, 8*S*, 9*R*, 10*S* and 20*R*. The absolute stereochemistry for the **A**-, **B**- and **C**-ring moieties was same as in (+)-puupehenone (**6**).¹³

Compound (**2**) was obtained as an inseparable mixture of two diastereoisomers (10:9). In the ¹H or ¹³C

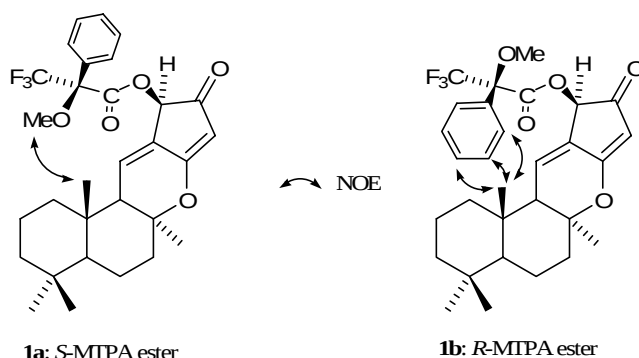


Figure 3. Key NOEs observed for the ester moieties of *S*- and *R*-MIPA esters (**1a** and **1b**).

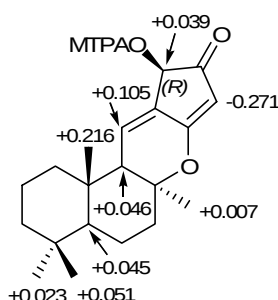


Figure 4. $\Delta\delta$ values ($\delta_S - \delta_R$ ppm) for the MIPA esters (**1a** and **1b**).

NMR spectra, some signals were overlapped and others appeared in pairs. The intensity ratio of signals that formed pairs could change, which indicated that two diastereoisomers were at equilibrium in CDCl_3 solution. The ^{13}C NMR (Table 1) and HRESIMS [m/z 355.1910 ($\text{M} + \text{Na}$) $^+$, calcd for $\text{C}_{20}\text{H}_{28}\text{O}_4\text{Na}$, 355.1885] data revealed that **2** had a formula of $\text{C}_{20}\text{H}_{28}\text{O}_4$. The ^1H NMR signals in the aliphatic region were almost identical to those of **1** or puupehenone (**6**). The main differences in the chemical shifts between **1** and **2** were seen for C19 (δ_{C} 202.1 in **1**, δ_{C} 166.6 in **2**) and the oxygenated methine at C20 [δ_{C} 70.8 d, δ_{H} 4.60 s in **1**; δ_{C} 94.1 d, δ_{H} 6.05 s in **2**]. A detailed analysis of 1D and 2D NMR data finally gave the structure of **2** with 5-hydroxy pentenolide in ring **D** as an oxidation product of **1** or **3**. Since the NOEs observed for the **A**-, **B**- and **C**-ring moieties in **2** resembled those described above for **1**, both compounds were assumed to possess an identical stereochemistry for the **A**-, **B**- and **C**-ring moieties. Compound (**2**) was an equilibrium mixture of epimers of each other at C20.

The structures of haterumadienone (**3**) and acetone adducts (**4** and **5**) were determined in the same way as for **1**, as described previously.¹⁰

CONCLUSION

Puupehenone (**6**) and its derivatives are an important group of marine metabolites because they display a wide range of properties including cytotoxic, antiviral, antitumor, antifungal, antimalarial, antituberculosis and immunomodulatory activities.¹⁻¹⁰ We recently isolated haterumadienone (**3**) and

acetone adducts (**4** and **5**) together with puupehenone (**6**) from the Okinawan sponge *Dysidea* sp.¹⁰ In this report, we have described the isolation, structure elucidation and biological activity of 20-Hydroxyhaterumadienone (**1**) and haterumadienelactol (**2**) which are related to haterumadienone (**3**), along with detailed experimental procedures for **3**, **4** and **5**. The metabolites (**1**, **2** and **3**) showed one less carbon of ring **D** than **6**. 20-Hydroxyhaterumadienone (**1**) and haterumadienelactol (**2**) would be formed by the oxidation of haterumadienone (**3**), which might be biosynthesized via benzylic acid rearrangement of the diketone form of puupehenone (**6**) followed by oxidative decarbonylation.¹⁰ Acetone adducts (**4** and **5**) appear to be artifacts derived from the trione hydrate (**10**) by aldol condensation with acetone in the extraction process. These acetone adducts are the most reasonable adducts because they have less electrostatic interactions between two ketonic groups (1, 3-diketones) than other adduct structures (1, 2-diketones).¹⁰

20-Hydroxyhaterumadienone (**1**), haterumadienelactol (**2**) and haterumadienone (**3**) inhibited the first cleavage of the fertilized sea urchin eggs by 9%, 47% and 40% at 5 ppm, respectively. Interestingly, the artificial acetone adducts (**4** and **5**) showed the potent toxicity comparable to that of puupehenone (**6**).¹⁰ Further chemical and biological studies on these metabolites are in progress in our laboratory.

EXPERIMENTAL

GENERAL ASPECTS

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/IR-300 spectrometer, respectively. The ¹H, ¹³C, and 2D NMR spectra were recorded on a JEOL α -500 spectrometer, and ¹H and ¹³C chemical shifts were referenced to the solvent peaks [δ_{H} 7.24 and δ_{C} 77.0 in CDCl₃]. 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 x 250 mm). Analytical TLC was performed using Kieselgel 60 F₂₅₄ DC-fertigplatten (Merck). All solvents used were reagent grade.

ISOLATION.

The yellowish sponge *Dysidea* sp. (750 g, wet weight) was extracted with acetone (1.5 L) twice. After filtration, the extracts were concentrated *in vacuo* to give an acetone extract. The acetone extract was partitioned between H₂O (200 mL) and EtOAc (300 mL x 2). The EtOAc extract (5.1 g) completely inhibited the first cell division of fertilized sea urchin eggs at 20 ppm. The toxic EtOAc-soluble material was triturated with hexanes. The hexane-insoluble part (3.1 g) 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 $\rightarrow$ 1:1, 300 mL $\rightarrow$ 1:3, 300 mL) and then EtOAc with increasing proportions of MeOH [EtOAc (300 mL) $\rightarrow$ EtOAc/MeOH (9:1, 200 mL $\rightarrow$ 7:1, 200 mL)] to give 23 fractions. Fractions 13-16 were combined and concentrated under reduced pressure. The resultant residue (0.65 g) was further chromatographed on ODS (33 g) using MeOH (200 mL) and 5% EtOAc in MeOH (100 mL) to give 14 fractions. The fourth fraction (80 mg) was subjected to reversed-phase HPLC on ODS using H₂O-MeOH (1:7) to give 10 fractions. The fourth and fifth fractions (14 mg each) were purified by successive reversed-phase HPLC on ODS using MeOH-H₂O (7:1) to afford acetone adducts (**4**; 2.5 mg and **5**; 3.0 mg). The sixth fraction was subjected to further separation by HPLC on Si60 using hexanes-EtOAc (1:1) to yield **1** (6.1 mg), **2** (5.2 mg), and **9** (3.1 mg). The hexane-soluble part (4.5 g) was first chromatographed on Si gel using hexanes with increasing proportions of EtOAc [hexanes (500 mL) $\rightarrow$ hexanes/EtOAc (5:1, 500 mL $\rightarrow$ 3:1, 500 mL $\rightarrow$ 1:1, 500 mL $\rightarrow$ 1:3, 500 mL) and then EtOAc with increasing proportions of MeOH [EtOAc (300 mL) $\rightarrow$ EtOAc/MeOH (9:1, 300 mL $\rightarrow$ 7:1, 300 mL)] to give 10 fractions. The third fraction (2.2 g) was partitioned between hexanes-EtOAc (4:1) (300 mL) and MeOH-H₂O (4:1) (300 mL) and the aqueous layer was concentrated under vacuum. The residual oil (1.2 g) was further chromatographed on Si gel using hexanes-EtOAc (4:1) to give **6** (369 mg) and a mixture of **3**, **7** and **8**. The mixture (150 mg) was subjected to reversed-phase HPLC on ODS using MeOH to give 3 fractions. The first fraction (70 mg) was further purified by reversed-phase HPLC on ODS using MeOH-H₂O (9:1) to afford **6** (6.2 mg), **7** (4.9 mg) and a fraction (25.5 mg) containing **8**. The fraction (25.5 mg) containing **8** was finally purified by reversed-phase HPLC on ODS using MeOH-H₂O (8.5:1.5) to afford **8** (5.3 mg). The fourth fraction (250 mg) from the first column separation of the hexane-soluble part was first subjected to separation by preparative TLC [hexanes-EtOAc (6:1)] to give a fraction that contained **3**. The fraction containing **3** was then purified by reversed-phase HPLC on ODS using MeOH to afford **3** (14.0 mg).

20-Hydroxyhaterumadienone (1) Colorless oil; $[\alpha]_D -94^\circ$ (*c* 0.28 CHCl₃); UV (MeOH) $\lambda_{\max}$ 284 (ϵ 20000) nm; FT IR $\nu_{\max}$ (film) 3314, 2926, 1660, 1594, 1419 and 1164 cm⁻¹; ¹H and ¹³C NMR (CDCl₃) data are listed in Table 1; HRESIMS *m/z* 339.1947 (M + Na)⁺ (calcd for C₂₀H₂₈O₃ Na, 339.1936).

Haterumadienelactol (2): Colorless oil; $[\alpha]_D +9.8^\circ$ (*c* 0.10 CHCl₃); UV (MeOH) $\lambda_{\max}$ 288 (ϵ 18000) nm; FT IR $\nu_{\max}$ (film) 3355, 2925, 1685, 1560, 1404 and 1186 cm⁻¹; ¹H and ¹³C NMR (CDCl₃) data are listed in Table 1; HRESIMS *m/z* 355.1910 (M + Na)⁺ (calcd for C₂₀H₂₈ O₄Na, 355.1885).

Haterumadienone (3): Colorless oil; $[\alpha]_D -57^\circ$ (*c* 0.14 CHCl₃); UV (MeOH) $\lambda_{\max}$ 282 (ϵ 23000) nm; FT IR $\nu_{\max}$ (film) 2920, 1696, 1573, 1405 and 1175 cm⁻¹; ¹H and ¹³C NMR (CDCl₃) data are listed in Table 1;

HRESIMS m/z 323.2017 ($M + Na$)⁺ (calcd for C₂₀H₂₈O₂Na, 323.1988).

Acetone adduct (4) (polar): Colorless oil; [α]_D -76° (*c* 0.12 CHCl₃); FT/IR (film) $\nu_{\max}$ 3400, 1730, 1260, 1240 cm⁻¹; UV $\lambda_{\max}$ 277 (ϵ 15000) nm; ¹H and ¹³C NMR (CDCl₃) data were described in the earlier paper;¹⁰ HRESIMS m/z 423.2154 ($M + Na$)⁺ (calcd for C₂₄H₃₂O₅Na, 423.2149).

Acetone adduct (5) (less polar): Colorless oil; [α]_D +183° (*c* 0.16 CHCl₃); FT/IR (film) $\nu_{\max}$ 3400, 1730, 1260, 1240 cm⁻¹; UV $\lambda_{\max}$ 277 (ϵ 15000) nm; ¹H and ¹³C NMR (CDCl₃) data were described in the earlier paper;¹⁰ HRESIMS m/z 423.2207 ($M + Na$)⁺ (calcd for C₂₄H₃₂O₅Na, 423.2149).

Esterification of 1. To a solution of 20-hydroxyhaterumadienone (**1**, 0.5 mg, 1.6 μ mol) in pyridine (0.1 mL) was added (-)-MTPA chloride (24 mg, 96 μ mol). The mixture was stirred at rt for 2 h and quenched with methanol (0.2 mL). H₂O (0.2 mL) was then added, and the mixture was extracted with EtOAc (1 mL x 3). The organic phase was dried (Na₂SO₄) and concentrated *in vacuo*. The residual oil was purified by preparative TLC [*R*_f=0.2, hexanes-EtOAc (6: 1)] to give the (*S*)-MTPA ester (**1a**, 0.6 mg, 70%). Using the same procedure as described above, (*R*)-MTPA ester (**1b**, 0.6 mg, 70%) was obtained from the reaction between (+)-MTPA chloride (32 mg, 128 μ mol) and **1** (0.5 mg, 1.6 μ mol) in pyridine (0.1 mL). **1a**: ¹H NMR (500 MHz, CDCl₃) δ 7.62 (2H, m), 7.40 (3H, m), 6.13 (1H, d, *J*=6.5), 5.67 (1H, s), 5.46 (1H, s), 3.54 (3H, s), 2.23 (1H, br d, *J*=14.5 Hz), 1.97 (1H, d, *J*=6.5), 1.64 (1H, td, *J*=4.8, 14.5 Hz), 1.51 (1H, m), 1.50 (2H, m), 1.50 (1H, m), 1.42 (1H, m), 1.26 (3H, s), 1.17 (1H, td, *J*=4.3, 13.9 Hz), 1.08 ((1H, dt, *J*=3.5, 13.5 Hz), 0.94 (1H, dd, *J*=4.8, 13.9 Hz), 0.90 (3H, s), 0.83 (3H, s), 0.80 (3H, s); LRESIMS m/z 533 ($M + H$)⁺ and 555 ($M + Na$)⁺. **1b**: ¹H NMR (500 MHz, CDCl₃) δ 7.63 (2H, m), 7.38 (3H, m), 6.02 (1H, d, *J*=6.5 Hz), 5.94 (1H, s), 5.42 (1H, s), 3.63 (3H, s), 2.20 (1H, br d, *J*=14.5 Hz), 1.92 (1H, d, *J*=6.5 Hz), 1.61 (1H, td, *J*=4.8, 14.5 Hz), 1.45 (1H, m), 1.43 (1H, m), 1.39 (2H, m), 1.27 (2H, m), 1.26 (3H, s), 1.14 (1H, td, *J*=4.3, 13.8 Hz), 0.97 ((1H, dt, *J*=3.5, 13.5 Hz), 0.91 (1H, m), 0.88 (3H, s), 0.78 (3H, s), 0.58 (3H, s); LRESIMS m/z 533 ($M + H$)⁺ and 555 ($M + Na$)⁺.

ACKNOWLEDGEMENT

This work supported in part by a grant from the Cabinet Office of Japan (Research Project by Okinawa Development and Promotion Bureau).

REFERENCES

1. B. N. Rabi, H. P. Perzanowski, R. A. Ross, T. R. Erdman, and P. J. Scheuer, *Pure & Appl. Chem.*, 1979, **51**, 1893.
2. P. Amade, L. Chevelot, H. P. Perzanowski, and P. J. Scheuer, *Helv. Chim. Acta*, 1983, **66**, 1672.

3. M. T. Hamann, P. J. Scheuer, and M. Kelly-Borger, *J. Org. Chem.*, 1993, **58**, 6565.
4. S. S. Nasu, B. K. S. Yeung, M. T. Hamann, P. J. Scheuer, M. Kelly-Borges, and K. Coins, *J. Org. Chem.* 1995, **60**, 7290.
5. M.-L. Bourguet-Kondracki, C. Debitus, and M. Guyot, *Tetrahedron Lett.*, 1996, **37**, 3861.
6. J. K. Zjawiony, P. Bartyzel, and M. T. Hamann, *J. Nat. Prod.*, 1998, **61**, 1502.
7. K. A. El Sayed, P. Bartyzel, X. Shen, T. L. Perry, J. K. Zjawiony, and M. T. Hamann, *Tetrahedron*, 2000, **56**, 949.
8. I. C. Pina, M. L. Sanders, and P. Crews, *J. Nat. Prod.*, 2003, **66**, 2.
9. T. Amagata, S. Whitman, T. A. Jhonson, C. C. Sressman, C. P. Loo, E. Lobkovsky, J. Clardy, P. Crews, and T. R. Holman, *J. Nat. Prod.*, 2003, **66**, 230.
10. K. Ueda, T. Ueta, E. R. O. Siwu, M. Kita, and D. Uemura, *Chem. Lett.*, 2005, **34**, 1530.
11. I. Ohtani, T. Kusumi, Y. Kashmann, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
12. J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **96**, 512.
13. S. Urban and R. J. Capon, *J. Nat. Prod.*, 1996, **59**, 900.