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A New α,β,γ,δ-Unsaturated Carboxylic Acid and Three New Cyclic Peroxides From the Marine Sponge, *Monotria japonica*, Which Selectively Lyse Starfish Oocytes Without Affecting Nuclear Morphology

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Abstract—The marine sponge *Monotria japonica* contains cytolytic constituents, which have been fractionated to a new α,β,γ,δ-unsaturated carboxylic acid designated monotriajaponide A (**1**) and three new cyclic peroxides designated monotriajaponides B (**2**), C (**3**), and D (**4**), in addition to a known peroxide (**5**) and a known α,β-unsaturated ester (**6**). The structures were determined on the basis of spectroscopic data. Compounds **1**–**5** lysed immature starfish (*Asterina pectinifera*) oocytes without affecting nuclear morphology at the minimum effective concentrations of 50, 6.3, 6.3, 6.3, and 13 μg/mL, respectively. On the other hand, compound **6**, at the minimum effective concentration of 25 μg/mL, lysed both oocyte's plasma membrane and the nuclear envelope.

Introduction

Oocytes in multi-cellular animals are generally large in size and the isolation of nuclei in a pure state is difficult, thereby the structural changes of nuclei during oocyte maturation and early embryogenesis being difficult to study. Therefore, chemicals which disperse cytoplasm keeping the integrity of nucleus intact would be useful for the study of the nuclear structure and functions during oocyte maturation and early embryogenesis. In the course of our search for biologically active compounds from marine organisms,[1–10] we have found that the MeOH extract of the marine sponge, *Monotria japonica* (Hoshino), collected off Oshima, Kagoshima Prefecture, Japan, lyses the plasma membrane of starfish (*Asterina pectinifera*) oocytes and embryos without affecting the nuclear structure. Bioassay-guided fractionation of the crude extract resulted in the isolation of a new α,β,γ,δ-unsaturated carboxylic acid, monotriajaponide A (**1**) and three new cyclic peroxides, namely monotriajaponides B (**2**), C (**3**), and D (**4**), as well as a known peroxide (**5**)[11] and a known α,β-unsaturated ester (**6**).[12] We describe herein the isolation, structure elucidation, and biological activities of these compounds.

Results and Discussion

The marine sponge *M. japonica* (87 g, wet weight) was immediately frozen after collection. The MeOH extract was partitioned between hexane and water. The hexane extract (2.4 g), which showed lysing activity against immature starfish (*A. pectinifera*) oocytes without affecting nuclear morphology, was subjected to column chromatographies on ODS and silica gel to afford monotriajaponides A (**1**; 1.0 mg), B (**2**; 4.9 mg), C (**3**; 548 mg), and D (**4**; 510 mg) together with known compounds **5** (25 mg) and **6** (90 mg) as viscous colorless oils.

Monotriajaponide A (**1**) had a molecular formula of $C_{18}H_{30}O_2$, as determined by the negative mode HRFABMS (*m*/*z* 277.2158 $[M–H]^-$, Δ −0.9 mmu). The IR absorption bands at 1690 cm^{-1} and 2400–3400 (broad) cm^{-1} implied the presence of a carboxyl group.

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Table 1. ^{1}H NMR data of monotriajaponides A–D (**1**–**4**) in $CDCl_3$[a]

No.	1	2	3	4
2a	5.90 d (15.8)	2.42 dd (15.8, 3.7)	2.42 dd (15.9, 3.7)	2.42 dd (15.9, 3.7)
2b		3.07 dd (15.8, 9.1)	3.06 dd (15.9, 9.1)	3.06 dd (15.9, 9.2)
3	7.70 d (15.8)	4.44 ddd (9.1, 4.9, 3.7)	4.45 ddd (9.1, 4.9, 3.7)	4.44 m
4		2.14 m	2.09 m	2.09 m
5a	5.42 d (10.4)	1.30 dd (13.4, 12.8)	1.25[b]	1.27[b]
5b		1.79 dd (13.4, 4.3)	1.69[b]	1.69 dd (13.4, 4.3)
6	2.57 m			
7a	1.28[b]	5.47 d (15.8)	5.12 br s	5.12 br s
7b	1.32[b]			
8	1.66 m	5.53 dt (15.8, 6.1)		
9a	4.98 dd (15.2, 9.1)	1.94 ddd (13.4, 7.3, 6.1)	1.78 dd (12.8, 7.9)	1.94 dd (2H, 12.8, 7.3)
9b		2.10 ddd (13.4, 6.1, 6.1)	2.03 dd (12.8, 6.4)	
10	5.26 dt (15.2, 6.1)	1.49[b]	1.60[b]	1.45 m
11a	2.00 m (2H)	1.11[b]	1.07 m	1.22 (2H)[b]
11b		1.32[b]	1.32[b]	
12	0.95 t (3H, 7.3)	1.33 (2H)[b]	1.31 (2H)[b]	1.24 (2H)[b]
13	2.27 q (2H, 7.3)	1.30 (2H)[b]	1.27 (2H)[b]	1.25 (2H)[b]
14	1.10 t (3H, 7.3)	0.89 t (3H, 7.3)	0.87 t (3H, 7.3)	0.87 t (3H, 6.7)
15a	1.19[b]	1.16[b]	1.15[b]	1.14[b]
15b	1.30[b]	1.23[b]	1.22[b]	1.21[b]
16	0.79 t (3H, 7.3)	0.92 t (3H, 7.3)	0.90 t (3H, 7.3)	0.90 t (3H, 7.3)
17a	1.21[b]	1.47 (2H)[b]	1.54 dq (13.4, 7.3)	1.53 dq (13.4, 7.3)
17b	1.42 m		1.61 dq (13.4, 7.3)	1.61 dq (13.4, 7.3)
18	0.80 t (3H, 7.3)	0.84 t (3H, 7.3)	0.86 t (3H, 7.3)	0.85 t (3H, 7.3)
19		0.87 d (3H, 6.7)	1.71 br s (3H)	1.71 br s (3H)
20			0.81 d (3H, 6.7)	1.26 (2H)[b]
21				0.83 t (3H, 7.3)

[a]Coupling constants, $J_{H\text{-}H}$ (in Hz), are given in parentheses.
[b]Overlapped with other signals.

The UV spectrum showed absorption maximum at 271 nm with the ε value of 19,200, suggesting the presence of an α,β,γ,δ-unsaturated carbonyl group.[13] Both ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) together with the DEPT spectrum revealed that **1** has 18 carbons consisting of a carbonyl carbon, two disubstituted double bonds, a trisubstituted double bond, two methines, five methylenes, and four methyl groups. Assignments of all the protonated carbons were made by the analysis of the ^{13}C–^{1}H COSY and HMQC spectra as shown in Table 2. Connectivities from C-2 to C-3 and from C-5 to C-12 were readily inferred from the ^{1}H–^{1}H COSY spectrum. Furthermore, H-6 correlated to the methylene protons (H_2-15) which in turn coupled to the methyl proton (H_3-16), and H-8 correlated to the methylene protons (H_2-17) which in turn coupled to the methyl protons

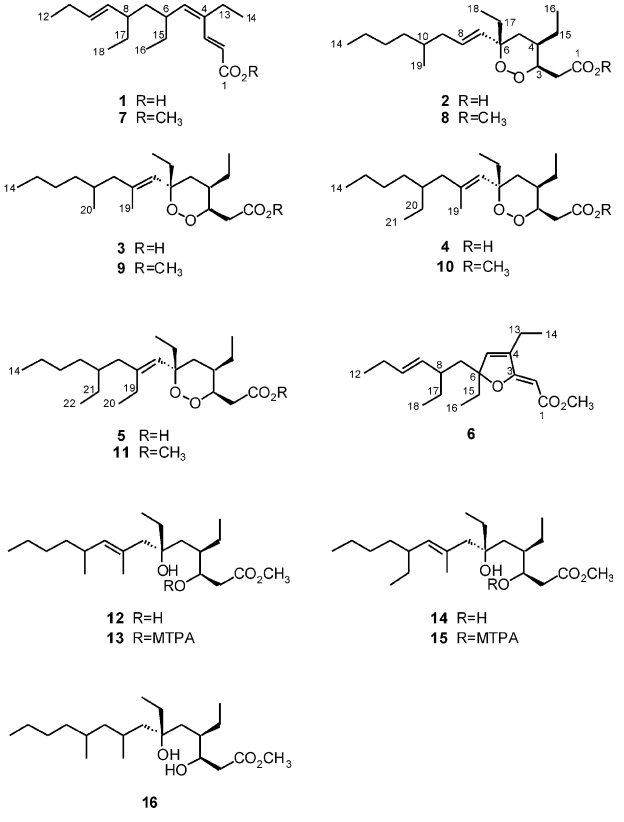

Table 2. ^{13}C NMR data of monotriajaponides A–D (**1**–**4**) in $CDCl_3$

No.	1	2	3	4
1	172.8 s	177.2 s	178.1 s	178.1 s
2	115.7 d	31.0 t	31.3 t	31.3 t
3	144.0 d	78.7 d	78.4 d	78.4 d
4	136.8 s	35.1 d	35.4 d	35.4 d
5	144.7 d	32.8 t	35.4 t	35.4 t
6	37.3 d	83.7 s	84.2 s	84.2 s
7	41.3 t	133.0 d	126.9 d	126.9 d
8	42.6 d	130.3 d	137.8 s	137.9 s
9	132.4 d	40.0 t	49.3 t	45.9 t
10	133.4 d	33.1 d	30.7 d	36.6 d
11	25.5 t	36.2 t	36.4 t	32.3 t
12	13.7 q	29.3 t	29.2 t	28.5 t
13	26.4 t	23.0 t	23.0 t	23.2 t
14	13.9 q	14.1 q	14.1 q	14.1 q
15	29.1 t[a]	25.0 t	24.9 t	24.9 t
16	11.7 q	11.0 q	11.0 q	11.0 q
17	29.2 t[a]	33.2 t	32.4 t	32.3 t
18	11.7 q	7.1 q	7.6 q	7.6 q
19		19.6 q	16.7 q	16.6 q
20			19.4 q	25.6 t
21				10.6 q

[a]Signals may be interchanged.

(H_3-18). This spectrum also revealed the presence of the isolated ethyl group C-13–C-14. Construction of the gross structure was achieved through HMBC experiments.[14] The correlations from H_3-14 to C-4, from H_2-13 to C-3, C-4 and C-5, from H-3 to C-5 and C-13, from H-5 to C-3 and C-13 connected the fragment C-2-C-3 to the C-5–C-12 fragment via C-4 and placed the ethyl group C-13–C-14 at C-4. Furthermore, the correlations of H-2 and H-3 to a carbon signal at δ_C 172.8 (C-1) placed the carbonyl group adjacent to C-2. Treatment of **1** with trimethylsilyldiazomethane gave the methyl ester **7**. In the ^{13}C NMR spectrum of **7**, the chemical shifts ascribable to a carbonyl carbon at δ_C 168.0 (C-1) and an sp^2 methine carbon at δ_C 116.7 (C-2) differed significantly from those of **1**, supporting the presence of the carboxyl group in **1**. Finally, the *E* geometry of Δ^2 and Δ^9 was assigned on the basis of the large coupling constants ($J_{2,3}$ = 15.8 Hz and $J_{9,10}$ = 15.2 Hz). NOE difference experiment data (Fig. 1) confirmed the geometry of Δ^2 and established the geometry of Δ^4. The H_2-13 methylene signals were enhanced upon irradiation of H-2 signal. Irradiation of the H-5 resulted in 3% enhancement of H_2-13 and 2% enhancement of H_3-14. These findings indicate that the geometry of Δ^4 is *Z*. Thus, the structure of monotriajaponide A (**1**) was determined to be (2*E*,4*Z*,9*E*)-4,6,8-triethyldodecatrienoic acid.

Monotriajaponide B (**2**) had a molecular formula of $C_{19}H_{34}O_4$, as determined by the negative mode HRFABMS (*m*/*z* 325.2388 $[M–H]^-$, Δ +0.9 mmu). The presence of a carboxyl group was implied by IR absorption bands at 1713 cm^{-1} and 2400–3400 (broad) cm^{-1}. Analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) as well as DEPT revealed that **2** has 19 carbons consisting of a carbonyl carbon, a disubstituted double bond, an oxygenated quaternary sp^3 carbon, an oxygenated sp^3 methine, eight methylenes, two methines, and four methyl groups. Considering that the molecular formula requires 3 degrees of unsaturation, **2** must contain a ring structure. Analysis of the ^{13}C–^1H COSY and HMQC spectra allowed the assignments of all the protonated carbons. From the ^1H–^1H COSY spectrum, the fragments C-2–C-5 and C-9–C-11 were readily inferred. Furthermore, correlations between H-4 and H_2-15, between H_2-15 and H_3-16, and between H-10 and H_3-19 were exhibited. The spectrum also revealed the presence of an isolated ethyl group C-17–C-18. HMBC experiments allowed the construction of the gross structure. The HMBC correlation from H_2-2 to the carbon signal at δ_C 177.2 placed the carbonyl group adjacent to C-2. Connectivities of C-5 and C-9 through C-6, C-7 and C-8 were deduced from HMBC cross peaks: H_2-5/C-6, H-7/C-6,C-8,C-9, H-8/C-6,C-7,C-9, and H_2-9/C-7,C-8. Connectivity of C-6 and C-18 via C-17 was justified by HMBC cross peaks H_2-17/C-5,C-6,C-7 and H_3-18/C-6. On the other hand, HMBC cross peaks H-11a/C-12,C-13 and H_3-14/C-12,C-13 revealed the connectivity of C-11 and C-14 through C-12 and C-13. All signals were able to be identified except for oxygen atoms at C-3 and at C-6, which allowed the closing of the cyclic peroxide ring. Treatment of **2** with trimethylsilyldiazomethane gave the methyl ester **8**. The presence of the carboxyl group in **2** was supported by the significant chemical shift (C-1; δ_C 172.2) in **8**. Finally, the *E* geometry of Δ^7 was assigned on the basis of its large coupling constant ($J_{7,8}$ = 15.8 Hz). The relative stereochemistry of the dioxane ring was elucidated on the basis of NOE difference experiments (Fig. 2) and the coupling constants ($J_{3,4}$ = 4.9 Hz, $J_{4,5a}$ = 12.8 Hz, and $J_{4,5b}$ = 4.3 Hz). The signal of the H-4 proton was enhanced upon irradiation of H-3. Irradiation of the H-5b proton signal resulted in enhancements of the signals of H-4, H-7, and H-8. These data placed the protons (H-3, H-4, H-5b, and H-7) on the same face of the dioxane ring and defined a chair conformation for the ring.

Monotriajaponide C (**3**) had a molecular formula of $C_{20}H_{36}O_4$, as determined by the negative mode HRFABMS (*m*/*z* 339.2540 $[M–H]^-$, Δ +0.5 mmu). The IR spectrum was nearly identical to those of **2**. The ^1H and ^{13}C NMR and DEPT spectra revealed that **3** is very similar to **2** except that **3** contains a broad singlet methyl proton signal at δ_H 1.71 and a broad singlet vinyl methine proton at δ_H 5.12 instead of vinyl methine proton signals at δ_H 5.47 (H-7) and at δ_H 5.53 (H-8) of **2** (Table 1). The HMQC and HMBC spectra allocated the methyl group to C-8 in **3**. NMR experiments completed the assignment of the structure of **3**. Treatment of **3** with trimethylsilyldiazomethane gave the methyl ester **9**. In the ^{13}C NMR spectrum of **9**, the chemical shift ascribable to a carbonyl carbon at δ_C 172.3 (C-1) differed significantly from those of **3**, supporting the presence of a carboxyl group in **3**. Finally, the observation of NOESY correlations between H-7 and H-9a and between H-7 and H-9b indicated the *E* geometry of Δ^7. The NOE difference experiments (Fig. 3) indicated the relative stereochemistry of the dioxane ring moiety of **3** is the same as that of **2**.

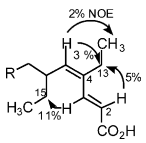

Figure 1. Key NOEs of the α,β,γ,δ-unsaturated carboxyl moiety in **1**.

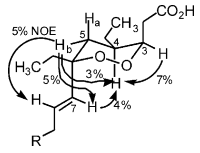

Figure 2. Key NOEs and relative stereochemistry of the dioxane ring moiety in **2**.

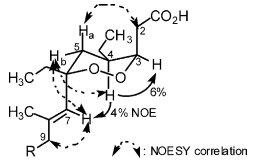

Figure 3. Key NOEs and relative stereochemistry of the dioxane ring moiety in **3**.

Monotriajaponide D (**4**) had a molecular formula of $C_{21}H_{38}O_4$, as determined by the negative mode HRFABMS (*m*/*z* 353.2690 $[M-H]^-$, Δ-0.2 mmu). The IR spectrum was nearly identical to those of **2** and **3**. The ^1H and ^{13}C NMR and DEPT spectra revealed that **4** is very similar to **3** except that **4** contains one methylene carbon (δ_C 25.6) and one triplet methyl proton signal (δ_H 0.83, $J=7.3$ Hz) instead of a doublet methyl proton signal (δ_H 0.81, $J=6.7$ Hz) of **3** (Tables 1 and 2). The HMQC and HMBC spectra placed an ethyl group at C-10 and completed the assignment of structure of **4**. Treatment of **4** with trimethylsilyldiazomethane gave the methyl ester **10**.[15] The chemical shift of the carbonyl carbon (C-1; δ_C 172.4) of **10** differed significantly from that of **4**, supporting the presence of the carboxyl group in **4**. The *E* geometry of Δ^7 was indicated by the observation of a NOESY correlation between H-7 and H_2-9. The deduced plane structure of monotriajaponide D (**4**) was identical to plakortide I, which was isolated as the methyl ester derivative from the marine sponge *Plakortis simplex*.[16] In the ^{13}C NMR of the methyl ester of monotriajaponide D (**10**), most of the resonances had virtually the same chemical shifts except for two carbons. The C-5 resonance in **10** was observed at δ_C 35.5, 3.2 ppm further downfield than the corresponding chemical shift in the methyl ester of plakortide I, and the C-17 resonance in **10** was observed at δ_C 32.4, 9.2 ppm further downfield than its δ_C 23.2 value in the methyl ester of plakortide I. These differences suggested that monotriajaponide D (**4**) is a diastereomer of plakortide I with inverted stereochemistry at C-6. This conclusion was further supported by measuring NOE difference experiments which are summarized in Figure 4. Furthermore, the NOE difference experiments and the NOESY spectrum indicated the relative stereochemistry of the dioxane ring moiety of **4** is the same as that of **3**.

The absolute stereochemistry of the dioxane rings of **2**–**4** was elucidated by using the modified MTPA method.[17] The methyl ester **9** was reduced with H_2/Pd-C[11] to afford the diol **12**. Although the Δ^7 was converted into the Δ^8 in this reduction, this should not affect the determination of the absolute configurations of the dioxane ring. Two aliquots of **12** were treated by (−)- and (+)-MTPA chloride in pyridine to afford MTPA esters **13a** and **13b**, respectively. The signs of $\Delta\delta$ (δ_S–δ_R) values for protons of H_2-2 and OCH_3 were positive, while those for protons from H-4 to H-9 and H_2-15 to H_3-19 were negative, as shown in Figure 5. In accordance with the modified Mosher model, the absolute configuration at C-3 was assigned as *R*. Therefore, the absolute stereochemistry of **3** was determined as 3*R*,4*R*,6*S*.

In the same way, as shown in Figure 6, the absolute stereochemistry of **4** was determined as 3*R*,4*R*,6*S*. Therefore, monotriajaponide D (**4**) is the C-6 epimer of plakortide I.

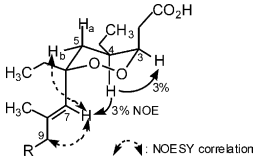

Figure 4. Key NOEs and relative stereochemistry of the dioxane ring moiety in **4**.

Table 3. Lysing effect of compounds **1**–**14** and **16** on immature starfish oocytes

Compd	Minimum effective concentration (μg/mL)	
	Oocyte lytic activity [a]	GV-disintegrating activity[b]
Monotriajaponide A (**1**)	50	>100
Monotriajaponide B (**2**)	6.3	>100
Monotriajaponide C (**3**)	6.3	>100
Monotriajaponide D (**4**)	6.3	>100
Cyclic peroxide **5**	13	>100
α,β-Unsaturated ester **6**	25	25
Methyl ester **7**	>100	>100
Methyl ester **8**	>100	>100
Methyl ester **9**	>100	>100
Methyl ester **10**	>100	>100
Methyl ester **11**	>100	>100
Diol **12**	>100	>100
Diol **14**	>100	>100
Saturated diol **16**	>100	>100

[a]At the indicated concentration, more than 50% oocytes lysed.
[b]At the indicated concentration, less than 50% oocytes contained the germinal vesicle.

Monotriajaponides B (**2**), C (**3**) and D (**4**) showed the positive Cotton effect at around 205 nm, due to the n→π* transition of carboxyl chromophore, indicating the C-3 chirality of **2** being the same as that of **3** and **4**. Thus, the absolute stereochemistry of the dioxane ring of **2** was determined as 3*R*,4*R*,6*S*.

Compound **5**, upon treatment with trimethylsilyldiazomethane, gave the methyl ester **11**, which was identical to the methyl ester of cyclic peroxide which have been isolated from the marine sponge *Plakortis* sp.[11] The spectral data of **6** were identical to those of an α,β-unsaturated ester isolated from the marine sponge *Plakortis halichondrioides*.[12]

Compounds **1**–**12**, **14**, and a saturated diol **16** derived from **3** were examined for the oocyte-lytic activity. As given in Table 3, cyclic peroxides **2**–**5** were potently active, and non-cyclic compound **1** exhibited weak activity. On the other hand, methyl esters **7**–**11**, **12**, **14**, and **16** did not affect the oocyte's morphology. Compounds **2**–**5**

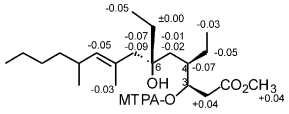

Figure 5. Δδ values for the MTPA esters **13a** and **13b**; Δδ (ppm) = δ_S–δ_R.

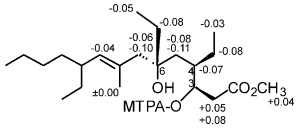

Figure 6. Δδ values for the MTPA esters **15a** and **15b**; Δδ (ppm) = δ_S–δ_R.

lysed oocytes, but remarkably, the germinal vesicle (nucleus) of the treated oocytes was kept intact for long periods in sea water. The germinal vesicle contains tetraploid chromosomes and is essential for normal fertilization of the egg and several developmentally important events in the embryo. Compound **6**, the methyl ester of an α,β-unsaturated carboxylic acid containing a furan ring, exhibited lytic activity, but it disrupted the germinal vesicle at the same concentration to disintegrate the plasma membrane. We consider that both functional groups, cyclic peroxide and carboxylic acid, are necessary to exhibit the specific activity to disperse cytoplasm keeping the integrity of nucleus intact. In order to examine the effect of compounds **3** and **4** on the nuclear structure of the embryo, oocytes were preloaded with Hoechst 33342, a fluorochrome which specifically stains DNA, and induced to mature by adding 1-methyladenine, a starfish maturation-inducing substance.[18] Fertilized eggs became 2-cell-stage embryos which were then placed in sea water containing various concentrations of compound **3** or **4**. The embryos treated with **3** at or above 6.3 μg/mL or **4** at or above 6.3 μg/mL halted embryonic development immediately after the treatment. The surface of the blastomeres was irregular and the color of the cytoplasm faded possibly due to the leakage of the cytoplasmic components. However, the integrity of nucleus was intact and chromatin was brightly fluorescent. Therefore, compounds **3** and **4** are cytolytic agents which allow the nuclear structure intact.

Experimental

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a JEOL LA 500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C). NMR chemical shifts were referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. FABMS, EIMS, HRFABMS, and HREIMS were measured on a JEOL SX102A spectrometer. UV and IR spectra were recorded on a Shimadzu UV-160A and a JASCO FT/IR-5300 spectrometer, respectively. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. CD spectra were recorded with a JASCO J-600 circular dichroic spectropolarimeter.

Biological materials

The marine sponge *M. japonica* (order Homosclerophorida, family Plakinidae) was collected off Ohshima, Kagoshima Prefecture, Japan (July 2001). The marine sponge was identified by Professor Patricia R. Bergquist, The University of Auckland, New Zealand. A voucher specimen is kept in the laboratory of one of the authors (S.O.).

Bioassays

Specimens of the starfish *A. pectinifera* were collected from coastal water off Japan during their breeding season and kept in sea water at 15 °C in laboratory aquaria. Oocytes and sperm were removed from ovarian and testicular fragments, respectively. Experiments were performed at 20 °C. Filtered sea water diluted to 90% (v/v) with distilled water was used throughout. Immature oocytes were placed in serially diluted sample solutions. 12 h later, the oocytes were examined under an inverted microscope and the percentage of the oocytes with an intact germinal vesicle and dispersed cytoplasm was determined. For the assay using embryos during early developmental stages, the presence of nuclei in the lysing cytoplasm was examined by fluorescence microscopy as follows. Ovarian fragments were immersed in sea water containing Hoechst 33324 (Calbiochem-Novabiochem, La Jolla, Carifonia, USA), a fluorochrome which selectively stains DNA, at the concentration of 10 μg/mL and 1 μM 1-methyladenine (Sigma, St. Louis, Missouri, USA), for 30 min, followed by transfer to sea water without any drugs. Maturing oocytes, i.e., eggs, released from ovarian fragments were collected by brief centrifugation, washed three times with sea water, and 40 min after the initiation of the treatment with 1-methyladenine, the eggs were fertilized by the addition of dilute sperm suspension. 10 min after insemination, fertilized eggs were washed three times with sea water and cultured in fresh sea water until the 2-cell-stage. Embryos were placed in serially diluted sample solutions. Twelve hours later, the embryos were examined for the presence of fluorescently stained nuclei by fluorescence microscopy using a V filter cassette. The integrity of the nuclear structure was examined under a phase contrast microscope.

Extraction and isolation

The marine sponge *M. japonica* (87 g, wet weight) was extracted with MeOH and the extract was concentrated. The concentrate was partitioned between hexane and water. The hexane extract (2.4 g) was chromatographed on a ODS column using 60→100% MeOH in H_2O as eluent to afford a 1:2 mixture (44 mg) of **1** and **2**, and pure compounds **3** (548 mg), **4** (510 mg), and **10** (65 mg) together with known compounds **5** (25 mg) and **6** (90 mg), as viscous colorless oils. A part of the mixture (24 mg) of **1** and **2** was further subjected to column chromatography on silica gel using 0→100% EtOAc in hexane as eluent to afford **1** (1.0 mg) and **2** (4.9 mg) as viscous colorless oils.

Monotriajaponide A (1). A viscous colorless oil; $[\alpha]_D^{25}$ +63° (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 nm (4.28); IR (film) ν_{max} 3400-2400 (COOH), 2963, 2930, 2874, 2853, 1690 (C=O), 1611, and 1287 cm^{-1}; ^{1}H and ^{13}C NMR: see Tables 1 and 2; (−)HRFABMS *m/z* 277.2158 [M–H]$^-$ (calcd for $C_{18}H_{29}O_2$, 277.2167).

Monotriajaponide B (2). A viscous colorless oil; $[\alpha]_D^{25}$ +127° (*c* 0.52, $CHCl_3$); IR (film) ν_{max} 3400–2400 (COOH), 2961, 2930, 2874, 1713 (C=O), 1462, and 1290 cm^{-1}; ^{1}H and ^{13}C NMR: see Tables 1 and 2; (−)HRFABMS *m/z* 325.2388 [M–H]$^-$ (calcd for $C_{19}H_{33}O_4$, 325.2379); CD $\Delta\varepsilon_{206}$ +3.7±0.3 (2.7×10^{-5} M, MeOH).

Monotriajaponide C (3). A viscous colorless oil; $[\alpha]_D^{25}$ +64° (*c* 0.60, $CHCl_3$); IR (film) ν_{max} 3400–2400 (COOH), 2959, 2926, 2876, 1715 (C=O), 1458, and 1289 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (−)HRFABMS *m/z* 339.2540 $[M-H]^-$ (calcd for $C_{20}H_{35}O_4$, 339.2535); CD $\Delta\varepsilon_{206}$ +2.5±0.3 (5.7×10^{-5} M, MeOH).

Monotriajaponide D (4). A viscous colorless oil; $[\alpha]_D^{25}$ +108° (*c* 0.89, $CHCl_3$); IR (film) ν_{max} 3400–2400 (COOH), 2959, 2930, 2876, 1713 (C=O), 1462, and 1289 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (−)HRFABMS *m/z* 353.2690 $[M-H]^-$ (calcd for $C_{21}H_{37}O_4$, 353.2692); CD $\Delta\varepsilon_{206}$ +4.7±0.3 (2.7×10^{-5} M, MeOH).

Methylation of 1. To a solution of **1** (1.3 mg) in MeOH (2 mL), was added 2 M trimethylsilyldiazomethane (0.5 mL) and left at room temperature for 30 min. After removing the solvent, the reaction mixture was purified by a short silica gel column chromatography (EtOAc–hexane, EtOAc: 2→10%) to afford **7** (1.2 mg). **7**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.75 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 51.4 (q, OCH_3), 116.7 (C-2), 168.0 (s, C-1); EIMS *m/z* 292 $[M]^+$ (20), 263 (41), 233 (17), 203 (42), 55 (100).

Methylation of 2–5. Following the method similar to that used in the methylation of **1**, compounds **2** (1.5 mg), **3** (4.7 mg), **4** (4.2 mg), and **5** (1.1 mg) were converted into **8** (0.8 mg), **9** (4.5 mg), **10** (3.8 mg), and **11** (1.1 mg), respectively. **8**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.71 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 51.9 (q, OCH_3), 172.2 (s, C-1); EIMS *m/z* 311 $[M-C_2H_5]^+$ (1), 295 (24), 237 (47), 43 (100). **9**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.71 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 51.8 (q, OCH_3), 172.3 (s, C-1); EIMS *m/z* 354 $[M]^+$ (2), 336 (6), 325 (85), 309 (100). **10**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.71 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 51.8 (q, OCH_3), 172.4 (s, C-1); EIMS *m/z* 368 $[M]^+$ (2), 350 (3), 339 (90), 323 (100). **11**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 3.71 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 51.9 (q, OCH_3), 172.4 (s, C-1); EIMS *m/z* 353 $[M-C_2H_5]^+$ (1), 337 (18), 323 (7), 279 (47), 57 (100).

Hydrogenation of 9. To a suspension of palladium carbon (10 mg) in EtOH (2 mL), was added a solution of **9** (4.4 mg) in EtOH (0.5 mL). The slurry was stirred at room temperature under 1 atm of H_2 for 1 h. Removal of the palladium catalyst by filtration through Celite afforded **12** (3.9 mg): ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.87 (3H, t, *J*=7.3 Hz, H_3-14), 0.91 (3H, t, *J*=6.1 Hz, H_3-18), 0.91 (3H, d, *J*=6.7 Hz, H_3-20), 0.94 (3H, t, *J*=7.3 Hz, H_3-16), 1.20 (3H, m, H-11a and H_2-12), 1.29 (2H, m, H_2-13), 1.30 (3H, m, H-5a and H_2-15), 1.32 (1H, m, H-11b), 1.48 (2H, m, H_2-17), 1.65 (1H, dd, *J*=15.2, 8.5 Hz, H-5b), 1.71 (3H, d, *J*=1.2 Hz, H_3-19), 1.97 (1H, m, H-4), 2.07 (1H, d, *J*=13.4 Hz, H-7a), 2.35 (1H, m, H-10), 2.36 (1H, m, H-7b), 2.38 (1H, m, H-2a), 2.54 (1H, dd, *J*=15.8, 10.4 Hz, H-2b), 3.71 (3H, s, OCH_3), 4.19 (1H, br d, *J*=10.4 Hz, H-3), 4.97 (1H, br d, *J*=9.1 Hz, H-9); EIMS *m/z* 338 $[M-H_2O]^+$ (1), 327 (1), 323 (2), 309 (100).

Preparation of MTPA ester of 12. To a solution of **12** (1.5 mg) in dry pyridine (200 μL), was added (−)-MTPA chloride (15 μL) and stirred at room temperature overnight. After removal of the solvent, the reaction mixture was purified by a short silica gel column chromatography (EtOAc–hexane, EtOAc: 5→20%) to afford (*S*)-MTPA ester **13a** (1.3 mg). In the same way, by using (+)-MTPA chloride, **12** (1.7 mg) was converted into (*R*)-MTPA ester **13b** (1.1 mg). **13a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.80 (3H, t, *J*=7.3 Hz, H_3-18), 0.87 (3H, t, *J*=7.3 Hz, H_3-14), 0.90 (3H, t, *J*=7.3 Hz, H_3-16), 0.92 (3H, d, *J*=6.7 Hz, H_3-20), 1.21 (4H, m, H-5a, H-11a, and H_2-12), 1.27 (2H, m, H_2-13), 1.29 (1H, m, H-5b), 1.38 (3H, m, H_2-15 and H-17a), 1.40 (1H, m, H-11b), 1.45 (1H, q, *J*=7.3 Hz, H-17b), 1.67 (3H, br s, H_3-19), 1.87 (1H, m, H-4), 2.02 (1H, d, *J*=13.4 Hz, H-7a), 2.05 (1H, d, *J*=13.4 Hz, H-7b), 2.34 (1H, m, H-10), 2.65 (1H, dd, *J*=15.8, 4.9 Hz, H-2a), 2.72 (1H, dd, *J*=15.8, 8.5 Hz, H-2b), 3.55 (3H, s, MTPA OCH_3), 3.66 (3H, s, OCH_3), 4.92 (1H, br d, *J*=9.1 Hz, H-9), 5.76 (1H, ddd, *J*=8.5, 4.9, 3.0 Hz, H-3), 7.39 and 7.55 (5H, m, MTPA phenyl protons); (+)FABMS *m/z* 595 $[M+Na]^+$, 573 $[M+H]^+$, 555 $[M-18+H]^+$. **13b**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.85 (3H, t, *J*=7.3 Hz, H_3-18), 0.87 (3H, t, *J*=7.3 Hz, H_3-14), 0.92 (3H, d, *J*=6.7 Hz, H_3-20), 0.94 (3H, d, *J*=6.7 Hz, H_3-20), 1.20 (2H, m, H_2-12), 1.22 (1H, m, H-5a), 1.23 (1H, m, H-11a), 1.27 (2H, m, H_2-13), 1.31 (1H, m, H-5b), 1.43 (2H, m, H_2-15), 1.45 (2H, q, *J*=7.3 Hz, H_2-17), 1.70 (3H, d, *J*=1.2 Hz, H_3-19), 1.94 (1H, m, H-4), 2.09 (1H, d, *J*=13.4 Hz, H-7a), 2.14 (1H, d, *J*=13.4 Hz, H-7b), 2.34 (1H, m, H-10), 2.61 (1H, dd, *J*=15.8, 4.9 Hz, H-2a), 2.68 (1H, dd, *J*=15.8, 8.5 Hz, H-2b), 3.50 (3H, s, MTPA OCH_3), 3.62 (3H, s, OCH_3), 4.97 (1H, br d, *J*=9.1 Hz, H-9), 5.79 (1H, ddd, *J*=8.5, 4.9, 3.0 Hz, H-3), 7.40 and 7.55 (5H, m, MTPA phenyl protons); (+)FABMS *m/z* 595 $[M+Na]^+$, 573 $[M+H]^+$, 555 $[M-18+H]^+$.

Hydrogenation of 10. Following the method similar to that used in the hydrogenation of **9**, methyl ester **10** (3.8 mg) was converted into **14** (3.4 mg). ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.83 (3H, t, *J*=7.3 Hz, H_3-21), 0.87 (3H, t, *J*=6.1 Hz, H_3-14), 0.92 (3H, t, *J*=7.3 Hz, H_3-18), 0.94 (3H, t, *J*=7.3 Hz, H_3-16), 1.14 (1H, m, H-11a), 1.15 (1H, m, H-20a), 1.16 (1H, m, H-12a), 1.27 (1H, m, H-12b), 1.31 (2H, m, H_2-13), 1.33 (2H, m, H_2-15), 1.32 (1H, m, H-5a), 1.39 (1H, m, H-11b), 1.43 (1H, m, H-20b), 1.48 (2H, m, H_2-17), 1.66 (1H, dd, *J*=15.2, 8.5 Hz, H-5b), 1.71 (3H, d, *J*=1.2 Hz, H_3-19), 1.97 (1H, m, H-4), 2.10 (1H, d, *J*=13.4 Hz, H-7a), 2.14 (1H, m, H-10), 2.36 (1H, m, H-2a), 2.39 (1H, m, H-7b), 2.54 (1H, dd, *J*=15.8, 10.4 Hz, H-2b), 3.71 (3H, br s, OCH_3), 4.19 (1H, br d, *J*=9.8 Hz, H-3), 4.88 (1H, br d, *J*=9.8 Hz, H-9); EIMS *m/z* 352 $[M-H_2O]^+$ (2), 341 (1), 337 (4), 323 (100).

Preparation of MTPA ester of 14. Following the method similar to that used in the preparation of MTPA ester of **12**, by using (−)-MTPA chloride, methyl ester of **14** (1.2 mg) was converted into (*S*)-MTPA ester **15a** (1.0 mg). In the same way, by using (+)-MTPA chloride, **14** (1.4 mg) was converted into (*R*)-MTPA ester **15b** (0.9 mg). **15a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H

0.80 (3H, t, $J=7.3$ Hz, H3-18), 0.82 (3H, t, $J=7.3$ Hz, H_3-21), 0.87 (3H, t, $J=7.3$ Hz, H_3-14), 0.91 (3H, d, $J=7.3$ Hz, H_3-16), 1.15 (2H, m, H-11a and H-20a), 1.21 (3H, m, H-5a and H_2-12), 1.28 (2H, m, H_2-13), 1.31 (2H, m, H_2-15), 1.38 (2H, m, H_2-17), 1.40 (2H, m, H-11b and H-20b), 1.41 (1H, m, H-5b), 1.67 (3H, br s, H_3-19), 1.88 (1H, m, H-4), 2.06 (1H, d, $J=13.4$ Hz, H-7a), 2.09 (1H, d, $J=13.4$ Hz, H-7b), 2.17 (1H, m, H-10), 2.65 (1H, dd, $J=15.8$, 4.9 Hz, H-2a), 2.75 (1H, dd, $J=15.8$, 8.5 Hz, H-2b), 3.55 (3H, s, MTPA OCH_3), 3.66 (3H, s, OCH_3), 4.85 (1H, br d, $J=9.8$ Hz, H-9), 5.76 (1H, ddd, $J=8.5$, 4.9, 3.0 Hz, H-3), 7.39 and 7.55 (5H, m, MTPA phenyl protons); (+)FABMS m/z 609 $[M+Na]^+$, 587 $[M+H]^+$, 569 $[M-18+H]^+$. **15b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.83 (3H, t, $J=7.3$ Hz, H_3-21), 0.85 (3H, t, $J=7.3$ Hz, H_3-18), 0.87 (3H, d, $J=7.3$ Hz, H_3-14), 0.94 (3H, d, $J=7.3$ Hz, H_3-16), 1.15 (1H, m, H-20a), 1.16 (1H, m, H-11a), 1.21 (1H, m, H_2-12), 1.29 (3H, m, H-5a and H_2-13), 1.39 (3H, m, H-11b and H_2-15), 1.41 (1H, m, H-20b), 1.46 (2H, m, H_2-17), 1.52 (1H, m, H-5b), 1.70 (3H, br s, H_3-19), 1.95 (1H, m, H-4), 2.12 (1H, d, $J=13.4$ Hz, H-7a), 2.15 (1H, m, H-10), 2.19 (1H, d, $J=13.4$ Hz, H-7b), 2.60 (1H, dd, $J=15.8$, 4.9 Hz, H-2a), 2.67 (1H, dd, $J=15.8$, 8.5 Hz, H-2b), 3.50 (3H, s, MTPA OCH_3), 3.62 (3H, s, OCH_3), 4.89 (1H, br d, $J=9.8$ Hz, H-9), 5.79 (1H, ddd, $J=8.5$, 4.9, 3.0 Hz, H-3), 7.40 and 7.55 (5H, m, MTPA phenyl protons); (+)FABMS m/z 609 $[M+Na]^+$, 587 $[M+H]^+$, 569 $[M-18+H]^+$.

Preparation of 16. To a suspension of palladium carbon (10 mg) in EtOH (2 mL), was added a solution of **9** (7.2 mg) in EtOH (0.5 mL). The slurry was stirred at room temperature under 1 atm of H_2 over night. Removal of the palladium catalyst by filtration through Celite afforded **16** (5.5 mg). **16**: EIMS m/z 311 $[M-C_2H_5-H_2O]^+$.

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15. Compound **10** was also isolated by the first ODS column chromatography as a new compound together with compounds **1–6**. Natural **10**: a viscous colorless oil; $[\alpha]_D^{25}$ +71° (*c* 1.31, $CHCl_3$); IR (film) ν_{max} 2959, 2926, 2857, 1744 (C=O), 1462, and 1163 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.84 (3H, t, $J=7.3$ Hz, H_3-21), 0.85 (3H, t, $J=7.3$ Hz, H_3-18), 0.87 (3H, t, $J=6.7$ Hz, H_3-14), 0.90 t (3H, $J=7.3$ Hz, H_3-16), 1.15 (1H, m, H-15a), 1.21 (1H, m, H-15b), 1.25 (5H, m, H-5a, H_2-11, and H_2-12), 1.27 (2H, m, H_2-13), 1.29 (2H, m, H_2-20), 1.45 (1H, m, H-10), 1.56 (1H, m, H-17a), 1.62 (1H, m, H-17b), 1.71 (3H, br s, H_3-19), 1.71 (1H, m, H-5b), 1.94 (2H, dd, $J=7.3$, 3.0 Hz, H_2-9), 2.09 (1H, m, H-4), 2.38 (1H, dd, $J=15.8$, 3.7 Hz, H-2a), 3.03 (1H, dd, $J=15.8$, 9.1 Hz, H-2b), 3.70 (3H, s, OCH_3), 4.46 (1H, ddd, $J=9.1$, 4.9, 3.7 Hz, H-3), 5.14 (1H, br s, H-7); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 7.6 (q, C-18), 10.6 (q, C-21), 11.0 (q, C-16), 14.1 (q, C-14), 16.7 (q, C-19), 23.2 (t, C-13), 25.0 (t, C-15), 25.6 (t, C-20), 28.6 (t, C-12), 31.3 (t, C-2), 32.3 (t, C-11), 32.4 (t, C-17), 35.4 (d, C-4), 35.4 (t, C-5), 36.6 (d, C-10), 46.0 (t, C-9), 51.8 (q, OCH_3), 78.7 (d, C-3), 84.1 (s, C-6), 127.1 (d, C-7), 137.7 (s, C-8), 172.3 (s, C-1); EIMS m/z 368 $[M]^+$ (1), 353 (7), 339 (13), 323 (58), 307 (18), 293 (39), 279 (14), 265 (31), 199 (57), 181 (199), 83 (87); HREIMS m/z 368.2923 $[M]^+$(calcd for $C_{22}H_{40}O_4$, 368.2926); CD $\Delta\varepsilon_{206}$ $+2.7\pm0.3$ (3.5×10^{-5} M, MeOH).
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