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by Deiske Sumilat 20

Submission date: 20-Aug-2019 12:04PM (UTC+0700)
Submission ID: 1161638588
File name: 2015_DASumilat_Absolute.pdf (552.03K)
Word count: 5604
Character count: 24161

Bioorganic & Medicinal Chemistry 23 (2015) 797–802

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Absolute structures and bioactivities of eryspongins and eurydiene obtained from the marine sponge *Eryspongia* sp. collected at Iriomote Island

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ARTICLE INFO

Article history:
Received 21 November 2014
Revised 17 December 2014
Accepted 18 December 2014
Available online 29 December 2014

Keywords:
Eryspongin
Eurydiene
Marine sponge
Eryspongia sp.
PTP1B

ABSTRACT

Three unique sesquiterpenes, named eryspongins A–C (**1**–**3**), have been isolated from the marine sponge *Eryspongia* sp. The absolute configuration of **1** was assigned as (4*R*,6*R*,9*S*) by comparing its experimental Electronic Circular Dichroism (ECD) spectrum with the calculated ECD spectra of both enantiomers, and the absolute configurations of **2**, **3** and artifact **4** were suggested on the basis of that of **1** by assuming common biogenesis of **1**–**3**. These absolute configurations were opposite to those depicted in the previous communication. Further separation of the remaining fractions lead to the isolation of a new C_{11}-polyketide, named as eurydiene (**5**), together with a known C_{11}-polyketide, nakitriol (**6**). The structure of **5** was assigned on the basis of its spectroscopic data as a bicyclic alcohol with a diene side chain. Dehydroeryspongin A (**4**) inhibited protein tyrosine phosphatase 1B (PTP1B), an important target enzyme for the treatment of type II diabetes and obesity, with an IC_{50} value of 3.58 μM. Moreover, compound **4** did not inhibit the proliferation of human hepatoma Huh-7 cells at 100 μM. One of the locations in which PTP1B has been detected is hepatocytes. Compounds **1**–**3**, **5**, and **6** were not active against PTP1B. The growth of human colon (HCT-15) and T-cell lymphoma (Jurkat) cells was not disturbed by compounds **1**–**6**.

1. Introduction

Much attention has recently been paid to natural products obtained from marine organisms such as marine invertebrates, microorganisms, and algae. Of these organisms, natural products with unique structural features and potent biological activities have been isolated from marine sponges (Porifera).[1] Marine sponges belong to the genus *Eryspongia* have been shown to contain various kinds of secondary metabolites, including steroidal sulfates,[2] secosteroids,[3] hydroquinones,[4] sesquiterpene quinones,[5] and furanoterpenoids.[6] Over the course of our research on new useful metabolites from marine invertebrates and microorganisms, we have identified three new unique sesquiterpenes, eryspongins A–C (**1**–**3**) (Fig. 1), from the marine sponge *Eryspongia* sp. collected at Iriomote Island in Okinawa, Japan, and a bioactive derivative of **1**, dehydroeryspongin A (**4**), which inhibited the activity of protein tyrosine phosphatase (PTP) 1B, an important target for the treatment of type II diabetes and obesity.[7] Further separation of the remaining fractions yielded a new C_{11}-polyketide, named as eurydiene (**5**), and a known C_{11}-polyketide, nakitriol (**6**)[8] (Fig. 1).

Eryspongins possess a unique six- and eight-membered bicyclic skeleton, and only five natural products in this class of sesquiterpenes: pallescensin B (**7**),[9a] nakafuran-8 (**8**),[9b] 5-hydroxynakafuran-8 (**9**),[9c] 5-acetoxynakafuran-8 (**10**),[9c] and *O*-methyl nakafuran-8-lactone (**11**),[9d] have thus far been reported. The absolute configurations of **1**–**4** were tentatively assigned by comparing the optical rotations of **1**–**4** with those of known compounds. We herein reported the absolute stereochemistries of eryspongins determined by the calculated ECD experiment, structure elucidation of eurydiene (**5**), and bioactivities of compounds **1**–**6**.

2. Results and discussion

The EtOH extract of *Eryspongia* sp. was separated by an ODS column into eight fractions by stepwise elution with a mixture of

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http://dx.doi.org/10.1016/j.bmc.2014.12.049
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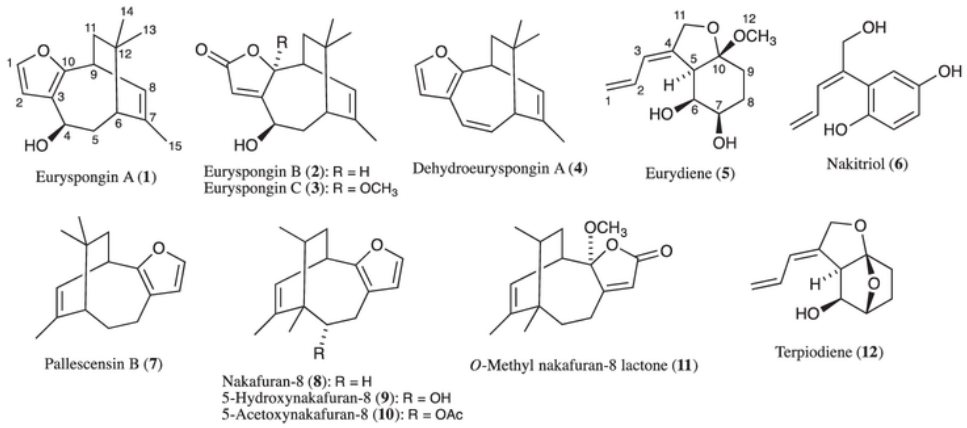

Figure 1. Structures of compounds **1–12**.

MeOH and H_2O. Compounds **1** (12.7 mg), **2** (0.9 mg), and **3** (1.6 mg) were isolated from the 80%, 60%, and 70% MeOH fractions, respectively, by repeated HPLC (ODS). The 50% MeOH fraction from an ODS column was subjected to preparative HPLC (ODS) to give compounds **5** (3.9 mg) and **6** (3.7 mg).

Compound **4** was obtained from eryspongin A (**1**) after the measurement of 2D NMR spectra in $CDCl_3$. The molecular formula, $C_{15}H_{18}O$, was determined from EIMS data, which suggested that **4** is a dehydro-derivative of **1**. The ^1H and ^{13}C NMR spectra of **4** showed two new sp^2 methine signals (δ_H 6.06, δ_C 122.1 and δ_H 5.71, δ_C 130.8) instead of oxygenated methine (δ_H 4.59, δ_C 65.9) and methylene signals (δ_H 2.16 and 2.27, δ_C 40.8) observed in the NMR spectra of **1**. These data revealed the dehydration occurred between C-4 and C-5. The *Z* configuration of the new double bond was assigned from the coupling constant (12.2 Hz) between H-4 and H-5. Thus, the structure of **4** was assigned as shown in Figure 1.

The structure of compound **6** was identified by comparison of the spectroscopic data of **6** with those of the reported values of nakitriol.[8]

2.1. Absolute configurations of eryspongins A–C (1–3) and dehydroeryspongin A (4)

The structures of eryspongins A–C (**1–3**) were elucidated from their HREIMS, UV, IR, and NMR data, as described previously.[7] The ^1H and ^{13}C NMR signals of **1–3** were assigned by an analysis of 2D NMR data (Table 1). ^1H–^1H COSY and HMBC data for **1–3** revealed the skeletal structures as shown in Figure 2.

The relative configurations of **1–3** were determined from NOESY data in $CDCl_3$ and 1D NOE difference experiments (C_6D_6),[7] and Figure 3 shows the stereostructures of **1–3** depicted by the Monte Carlo conformational analysis performed with an MMFF94 force field utilizing Spartan'08.[10] The absolute configurations of **1–3** were presumed by comparing the specific rotations of **1** and **2** with those of 5-hydroxynakafuran-8 (**9**)[9c] and *O*-methoxy nakafuran-8 lactone (**11**),[9d] respectively, since the application of the modified Mosher's method[11] was not successful.[7]

The absolute configurations of eryspongin A (**1**) was confirmed by comparison of the experimental Electronic Circular Dichroism (ECD) spectrum of **1** with the calculated ECD spectra for both

Table 1
^1H and ^{13}C NMR data for eryspongins A–C (**1–3**) in $CDCl_3$

Position	Eryspongin A (**1**)		Eryspongin B (**2**)		Eryspongin C (**3**)	
	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)
1	138.8	7.15 d (1.9)	172.5	–	166.8	–
2	109.3	6.37 d (1.9)	118.0	6.26 s	119.9	6.30 d (1.0)
3	122.6	–	176.1	–	170.1	–
4	65.9	4.59 dd (11.1, 4.8)	67.0	4.32 dd (10.6, 5.5)	65.7	4.32 ddd (10.0, 5.6, 1.0)
5a	40.8	2.27 ddd (14.0, 9.2, 4.8)	34.9	2.22 ddd (14.1, 8.6, 5.7)	35.6	2.22 ddd (14.0, 8.2, 5.8)
5b		2.16 ddd (13.9, 11.0, 1.1)		1.95 dd (13.9, 10.6)		1.98 dd (14.0, 10.6)
6	48.1	2.08 d (8.7)	47.2	1.89 d (8.8)	47.5	1.90 d (8.7)
7	141.5	–	142.5	–	140.4	–
8	120.2	5.74 dq (7.3, 1.4)	118.8	5.61 d (6.6)	119.4	5.60 dq (7.3, 1.0)
9	33.8	3.45 ddd (7.3, 6.0, 2.2)	33.9	2.96 br t	37.2	2.97 br t
10	150.1	–	83.5	4.85 s	109.4	–
10-OMe	–	–	–	–	51.1	3.23 s
11	44.2	1.56 dd (13.3, 6.0) 1.64 dd (13.3, 2.2)	30.2	1.25 m 1.35 dd (15.0, 9.5)	33.8	1.25 m 1.53 dd (15.5, 10.1)
12	33.4	–	34.0	–	33.1	–
13	36.3	0.78 s	29.3	0.93 s	29.8	0.92 s
14	30.1	0.90 s	33.7	0.95 s	34.7	0.96 s
15	23.5	1.87 d (1.4)	24.0	1.81 s	24.0	1.82 d (1.0)

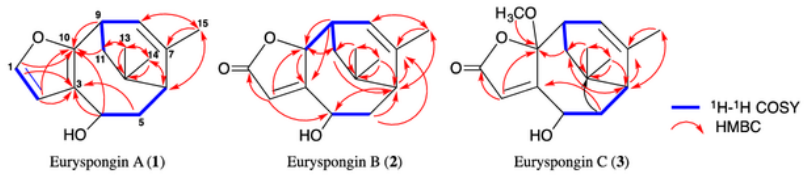

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations for eurypongins A–C (**1**–**3**).

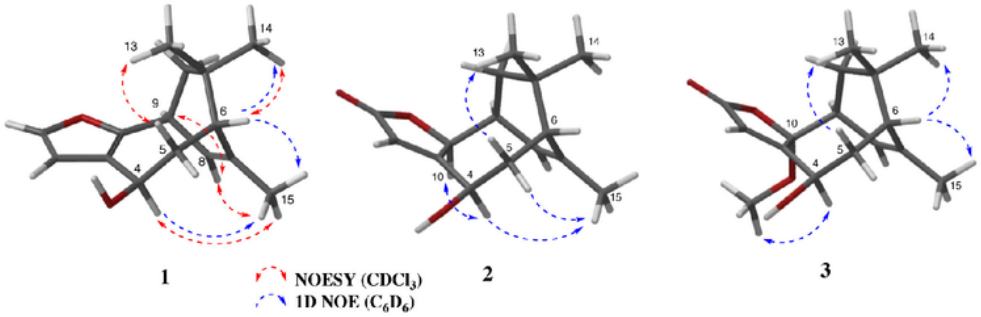

Figure 3. Stereostructures of eurypongins A–C (**1**–**3**).

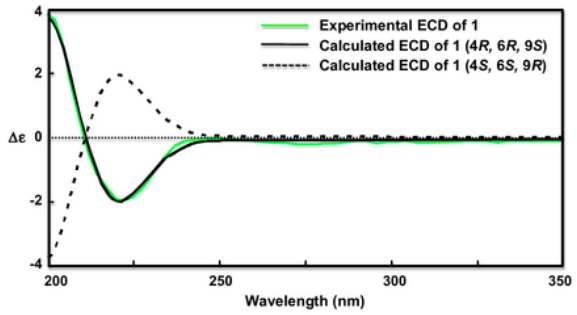

Figure 4. Experimental CD spectrum of eurypongin A (**1**) (green line) and calculated ECD spectra of **1** (black line) and its enantiomer (dashed line).

enantiomers of **1** (Fig. 4). The ECD spectra of (4*R*,6*R*,9*S*)-**1** (solid line) and (4*S*,6*S*,9*R*)-**1** (dashed line) were calculated for the energy-minimized structures based on the NOE data. The experimental ECD of **1** (green line) coincided with the calculated ECD spectrum of (4*R*,6*R*,9*S*)-isomer (solid line). Therefore, the absolute configuration of **1** was assigned as shown in Figure 1. The absolute configurations of **2** and **3** were deduced to be 4*R*, 6*R*, 9*S*, and 10*S* since compounds **1**–**3** were thought to be biosynthesized by the same pathway. Dehydroeurypongin A (**4**) was transformed from eurypongin A (**1**), and, therefore, the absolute configurations at the 6 and 9 positions of **4** were assigned as *R* and *S*, respectively.

2.2. Structure of eurydiene (5)

The molecular formula of eurydiene (**5**) was deduced to be $C_{12}H_{18}O_4$ from HREIMS and NMR data. The ^{1}H and ^{13}C NMR signals (Table 2) of **5** were assigned by analyzing DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, and **5** consisted of three sp^3 methylene, one sp^3 methine, one sp^3 oxygenated methyl, two sp^3 oxygenated methine, one sp^3 oxygenated quaternary (acetal), one sp^2 methylene, two sp^2 methine, and one sp^2 quaternary carbons. The ^{1}H–^{1}H COSY spectrum of **5** revealed two partial structures I and II, which were

Table 2
^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **5** (CD$_3$OD)

Position	Eurydiene (**5**)	
	δ_C	δ_H mult. (*J* in Hz)
1	115.5	(a) 4.99 ddd (10.6, 2.0, 1.0) (b) 5.07 ddd (17.0, 1.0, 1.0)
2	136.9	6.71 ddd (17.0, 10.6, 10.6)
3	123.7	6.03 d (10.6)
4	144.5	—
5	51.3	2.92 d (10.0)
6	74.5	3.21 dd (10.0, 2.4)
7	70.2	3.89 m
8	27.6	(a) 1.81 m (b) 1.56 dddd (14.1, 14.0, 5.0, 2.0)
9	24.1	(a) 1.89 ddd (13.9, 14.0, 5.0) (b) 2.01 ddd (13.9, 5.0, 2.0)
10	109.4	—
11	70.1	(a) 4.30 d (13.7) (b) 4.51 d (13.7)
12	48.5	3.18 s

connected by HMBC data (Fig. 5). The HMBC correlations from H-3 (δ 6.03) to C-5 (δ 51.3), H-5 (δ 2.92) to C-11 (δ 70.1), and from H-6 (δ 3.21) to C-4 (δ 144.5) proved the connection between C-4 and C-5. The positions of an acetal carbon and OMe group were assigned by the HMBC correlations to the acetal carbon at δ 109.4 (C-10) from H-5, H-6, H$_2$-8 (δ 1.56 and 1.81), H$_2$-9 (δ 1.89 and 2.01), H$_2$-11 (δ 4.30 and 4.51), and OMe (H$_3$-12, δ 3.18). Thus, the skeletal structure of eurydiene (**5**) was elucidated as shown in Figure 5.

The relative configuration of compound **5** was elucidated from NOESY data (Fig. 6). The NOESY correlations between H-5 (δ 2.92)/H-9a (δ 1.89), H-5/H-11a (δ 4.30), H-6 (δ 3.21)/H-7 (δ 3.89), H-7/H-9a, and H-9b (δ 2.01)/H$_3$-12 (δ 3.18) revealed the stereochemistry of the cyclohexane ring, which was the same as that of the related compound, terpiodiene (**12**), obtained from the Okinawan marine sponge *Terpios hoshinota*.[12] The orientation of the C-3 double bond in **5** was determined to be *E* from the NOESY correlation between H-3 (δ 6.03) and H$_2$-11 (δ 4.30 and 4.51). Figure 6 shows the relative stereostructure of eurydiene (**5**) obtained by the Monte Carlo conformational analyses[10] based on NOE data.

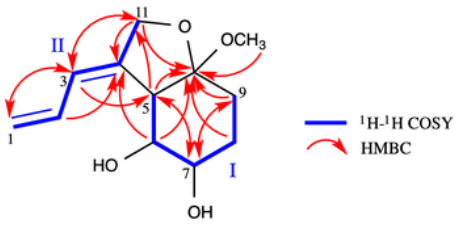

Figure 5. ^{1}H–^{1}H COSY and key HMBC correlations for eurydiene (**5**).

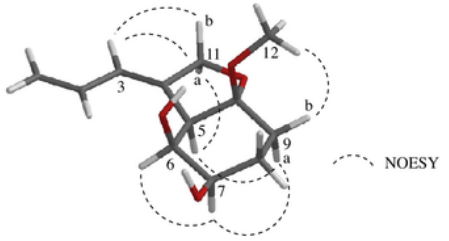

Figure 6. Stereostructure of eurydiene (**5**).

The absolute configuration of terpiodiene (**12**) was determined by the modified Mosher's method,[12] and, therefore, the specific rotation of **12** was compared with that of **5**. Eurydiene (**5**) ([α]$_D$ +82.3) and terpiodiene (**12**) ([α]$_D$ +46.0)[13] with known absolute configuration likely have the same absolute configuration because both showed the same signs of the specific rotations.

Eurydiene (**5**), nakitriol (**6**), and terpiodiene (**12**) belong to the cyclic C$_{11}$ polyketides, which have been isolated from marine organisms, such as ascidians, cyanobacteria, and marine sponges.[8,13–16] Most C$_{11}$ compounds were constructed from five acetates (C$_{10}$) by incorporating a C$_1$ unit or from six acetates (C$_{12}$) by eliminating CO$_2$.[15] Nakitriol (**6**) and terpiodiene (**12**) were assumed to have been biosynthesized from five acetates and a C$_1$ unit,[16] and eurydiene (**5**) will be biosynthesized by the same pathway as **6** and **12**.

2.3. Biological activity

Dehydroeuryspongin A (**4**) exhibited inhibitory activity against PTP1B (Table 3).[7] This enzyme is regarded as a key target for the treatment of type II diabetes and obesity because PTP1B plays an important role in the dephosphorylation of insulin and leptin receptors.[17] Nakafuran-8 (**8**) and *O*-methoxy nakafuran-8 lactone (**11**) were previously reported to inhibit PTP1B activity,[9d,18] whereas eurypongins A–C (**1–3**) did not. Therefore, an OH group at the C-4 position markedly reduced the activities of these compounds against PTP1B.[7] Eurydiene (**5**) and nakitriol (**6**) were not active against PTP1B at 40 and 50 μM, respectively (Table 3). Compounds **1–6** did not inhibit the proliferation of the two human cancer cell lines, HCT-15 (colon) and Jurkat (T-cell lymphoma), at 35–50 μM (Table 3). Moreover, compound **4** did not show cytotoxicity against human hepatoma Huh-7 cells at 100 μM. Huh-7 cells have been used in cell-based experiments to investigate the mechanisms of action of PTP1B inhibitors.[17] Therefore, dehydroeuryspongin (**4**) has potential as a drug candidate or lead compound for the development of a new type of PTP1B inhibitors.

Table 3
Bioactivities (IC$_{50}$, μM) of compounds **1–6** against PTP1B and three human cancer cell lines

Compound	PTP1B	Cytotoxicity		
		Huh-7	HCT-15	Jurkat
1	>40	>100	>40	>40
2	>40	nt[a]	>40	>40
3	>35	nt	>35	>35
4	3.58	>100	>45	>45
5	>40	nt	>40	>40
6	>50	nt	>50	>50
Oleanoic acid	1.17	nt	nt	nt

[a] nt: not tested.

3. Experimental section

3.1. General experimental procedure

EIMS was performed using a JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded on a JNM-AL-400 NMR spectrometer (JEOL) at 400 MHz for ^{1}H and 66 MHz for ^{13}C in $CDCl_3$ (δ_H 7.26, δ_C 77.0), C_6D_6 (δ_H 7.15), or CD_3OD (δ_H 3.31, δ_C 49.0). Optical rotations were measured with a digital polarimeter (P-2300; JASCO, Tokyo, Japan). UV spectra were recorded on a spectrophotometer (U-3310 UV–Visible spectrophotometer; Hitachi Ltd, Tokyo, Japan), CD spectrum on a spectrometer (J-720; JASCO), and IR spectra on a Fourier transform infrared spectrometer (FT-710; Horiba Ltd, Kyoto, Japan). Preparative HPLC was carried out using the L-6200 system (Hitachi Ltd, Tokyo, Japan).

3.2. Materials

Protein tyrosine phosphatase 1B (PTP1B) was purchased from Enzo Life Sciences (Farmingdale, NY). *p*-Nitrophenyl phosphate (*p*NPP) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) and other culture materials were purchased from Invitrogen (Carlsbad, CA, USA). Oleanolic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan). Plastic plates (96-well) were purchased from Corning Inc. (Corning, NY, USA). All other chemicals including organic solvents were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan).

3.3. Marine sponge and isolation of compounds 1–6

The marine sponge *Euryspongia* sp. was collected at Iriomote Island in Okinawa, Japan, by scuba diving in 2010. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University as 10-10-11=2-2.

The frozen sponge (306.6 g, wet weight) was thawed, cut into small pieces, and extracted with EtOH (1.0 L). The EtOH extract (1.1 g), after the evaporation of EtOH, was adsorbed on an ODS column (100 g). The column was eluted stepwise with 0%, 50%, 60%, 70%, 80%, 90%, and 100% MeOH in H_2O (each 400 mL). The 80% MeOH fraction (140.8 mg) was purified by preparative HPLC [column, PEGASIL ODS (Senshu Sci. i.d. 10 × 250 mm); solvent, MeOH/H_2O = 7:3; flow rate, 2.0 mL/min; detection, UV 220 nm] to give 12.7 mg of eurypongin A (**1**, t_R = 17.1 min). The 60% MeOH (12.7 mg) fraction gave 0.9 mg of eurypongin B (**2**, t_R = 23.1 min) by preparative HPLC (ODS, MeOH/H_2O = 65:35). The 70% MeOH fraction (84.6 mg) was purified by preparative HPLC (ODS, MeOH/H_2O = 7:3) and yielded 1.6 mg of eurypongin C (**3**, t_R = 24.2 min). The 50% MeOH fraction (44.0 mg) was separated by preparative HPLC (ODS, MeOH/H_2O = 1:1, 2.0 mL/min, UV 220 nm) to give 3.9 mg of eurydiene (**5**, t_R = 14.3 min) and 3.7 mg of nakitriol (**6**, t_R = 10.4 min).

3.4. Eryspongin A (1)

Colorless oils; $[\alpha]_D^{20}$ −33.9 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3402, 2357, 1630, 1385 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 220 (12850), 269 (8630); CD (CH_3CN) $\lambda_{extermum}$ nm ($\Delta\varepsilon$) 228 (−1.8); EIMS *m/z* 232 $[M]^+$; HREIMS *m/z* 232.1462 ([M]$^+$, calcd for $C_{15}H_{20}O_2$, 232.1463); ^1H NMR (C_6D_6) δ 7.05 (1H, d, *J* = 1.9 Hz), 6.51 (1H, d, *J* = 5 Hz), 5.51 (1H, d, *J* = 7.6 Hz), 4.45 (1H, dd, *J* = 10.7, 4.9 Hz), 3.45 (br t, *J* = 7.8 Hz), 2.08 (1H, ddd, *J* = , 10.7, 1.5 Hz), 1.99 (1H, ddd, *J* = 1 8.8, 4.9 Hz), 1.80 (1H, d, *J* = 8.8 Hz), 1.69 (1H, dd, *J* = 13.4, 1.7 Hz), 1.63 (3H, d, *J* = .5 Hz), 1.47 (1H, dd, *J* = 13.2, 6.3 Hz), 0.78 (3H, s), 0.72 (3H, s); ^1H and ^{13}C NMR ($CDCl_3$), see Table 1.

3.5. Eryspongin B (2)

Colorless oils; $[\alpha]_D^{20}$ +90.4 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3545, 1748, 1648, 1424 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 201 (20510), 220 (11310); EIMS *m/z* 248 $[M]^+$; HREIMS *m/z* 248.1401 ([M]$^+$; calcd for $C_{15}H_{20}O_3$, 248.1412); ^1H NMR (C_6D_6) δ 6.02 (1H, s), 5.02 (1H, d, *J* = 5.3 Hz), 4.25 (1H, s), 3.57 (1H, dd, *J* = 10.1, 5.8 Hz), 2 (1H, br t), 1.74 (1H, ddd, *J* = 14.5, 5.8 Hz), 1.56 (1H, dd, 12.6, 10.6 Hz), 1.37 (3H, s), 1.33 (1H, d, *J* = 8.7 Hz), 1.03 (1H, dd, *J* = 1 9.4 Hz), 0.86 (1H, d, *J* = 15.5 Hz), 0.66 (3H, s), 0.65 (3H, s); ^1H and ^{13}C NMR ($CDCl_3$), see Table 1.

3.6. Eryspongin C (3)

Colorless oils; $[\alpha]_D^{20}$ +52.2 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3468, 1760, 1647, 1427 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 201 (20600), 220 (11410); EIMS *m/z* 278 $[M]^+$; HREIMS *m/z* 278.1509 ([M]$^+$; calcd fo $C_{16}H_{22}O_4$, 278.1518); ^1H NMR (C_6D_6) δ 6.06 (1H, s), 5.54 (1H, d, *J* = 6.8 H 3.96 (1H, dd, *J* = 10.6, 5.8 Hz), 2.90 (1H, br t), 2.89 (3H, s), 1.7 H, m), 1.59 (1H, dd, *J* = 14.1, 10.4 Hz), 1.48 (3H, s), 1.40 (1H, d, *J* = 8.2 Hz), 1.2 H, dd, *J* = 15.9, 8.0 Hz), 0.93 (1H, d, *J* = 15.5 Hz), 0.68 (6H, s); ^1H and ^{13}C NMR ($CDCl_3$), see Table 1.

3.7. Dehydroeryspongin A (4)

Dehydroeryspongin A (**4**) was formed from eryspongin A (**1**) by dehydration in an NMR tube. Three days after the measurement of 2D NMR spectra of **1** in $CDCl_3$, the ^1H MR spectrum showed signals due to a dehydrated product. The ^1H and ^{13}C NMR spectra of this product revealed that the purity of **4** was high, that is, signals due to **1** were not detected. The solvent was e orated to afford compound **4**: pale yellow oils; $[\alpha]_D^{20}$ +55.9 (*c* 0.46, $CHCl_3$); UV (MeOH) λ_{max} nm (ε) 200 (17900), 280 (5480); EIMS *m/z* 214 $[M]^+$; HREIMS *m/z* 214.1365]$^+$; calcd for $C_{15}H_{18}O$, 214.1358); ^1H NMR ($CDCl_3$) δ 7.02 (1H, d, *J* = 1.5 Hz), 6.20 (1H, d, *J* = 1.9 Hz), 5 (1H, d, *J* = 12.2 Hz), 5.71 (1H, dd 12.2, 9.7 Hz), 5.67 (1H, dd, *J* = 7.3, 1.5 Hz), 3.30 (1H, t, *J* = 8.0 Hz), 2. 1H, d, *J* = 9.7 Hz), 1.90 (1H, d, *J* = 14.0 Hz), 1.81 (3H, = 1.4 Hz), 1.64 (1H, dd, *J* = 14.3, 9.4 Hz), 1.13 (3H, s), 0.97 (3H, s); ^{13}C NMR ($CDCl_3$) δ 161.9, 137.7, 133.0, 130.8, 122.1, 118.2, 114.4, 113.7, 51.7, 41.5, 39.6, 33.2, 32.4, 29.1, 23.4.

3.8. Eurydiene (5)

Colorless oils; $[\alpha]_D^{20}$ +82.3 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3402, 1607, 1438, 1073 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 201 (15450), 235 (16750); EIMS *m/z* 226 $[M]^+$; HREIMS *m/z* 226.1196 ([M]$^+$; calcd for $C_{12}H_{18}O_4$, 226.1205); ^1H and ^{13}C NMR (CD_3OD), see Table 2.

3.9. Nakitriol (6)

Pale yellow oils; UV (MeOH) λ_{max} nm (ε) 201 (14700), 220 (9390), 280 (3150); EIMS *m/z* 192 $[M]^+$; HREIMS *m/z* 192. ([M]$^+$; calcd for $C_{11}H_{12}O_3$ 2.0786); ^1H NMR (CD_3OD) δ 6.57 (1H, d, *J* = 8.7 Hz), 6.50 (1H, dd, *J* = .7, 2.9 Hz), 6.38 (1H, d, *J* = 2.9 Hz), 6.28 (1H, d, *J* = 10.5 Hz), 7 (1H, ddd, *J* = 16.7, 10.5, 9.9 Hz), 5.14 (1H, dd, *J* = 16.7, 2.2 Hz), 4.92 (1H, dd, *J* = 9.9, 2.2 Hz), 4.18 (2H, s); ^{13}C NMR (CD_3OD) δ 151.0, 148.5, 142.0, 135.6, 129.4, 127.2, 118.5, 117.7, 117.5, 116.4, 66.8.

3.10. Calculation of ECD

The most stable conformer of eryspongin A was predicted using Spartan'08[10] by a preliminary conformational analysis th the MMFF94 force field followed by geometry optimization using the density functional theory (DFT) with the B3LYP functional and 6-31G(d,p) basis set. The ECD spectrum in acetonitrile was calculated for the predicted most stable con er using Gaussian 03[19] by the time-dependent DFT (TDDFT) with the B3LYP functional and 6-31+G(d,p) basis set. The solvent effect was introduced by the polarizable continuum model (PCM). Ten low-lying excited states were calculated (corresponding to the wavelength region down to approximately 200 nm). The calculated spectrum was displayed using GaussView 5.0.9[20] with the peak half-width at half height being 0.333 eV. The calculated spectrum was shifted by −15 nm to match the experimental spectrum.

3.11. PTP1B inhibitory assay

PTP1B inhibitory activity was determined by measuring the rate of hydrolysis of the substrate, *p*NPP, according to a previously described method with a slight modi tion.[21] Briefly, PTP1B (100 μL of 0.5 μg/mL stock solution) in 50 mM citrate buffer (pH 6.0) containing 0.1 M NaCl, 1 mM dithiothreitol (DTT), and 1 mM *N,N,N′,N′*-ethylenediamine tetraacetate (EDTA) was added to each well of a 96-well plastic plate. A sample (2.0 μL in MeOH) was added to each wel make final concentrations from 0 to 35–45 μM and then incubated for 10 min at 37 °C. The reaction was initiated by the additio *p*NPP (100 μL of 4.0 mM stock solution) in the citrate buffer, incubated at 37 °C for 30 min, and the reaction was terminated by the ad on of 10 μL of a stop solution (10 M NaOH). The optical density of each well was measured at 405 nm using an MTP-500 microplate reader (Corona Electric Co., Ltd, Ibaraki, Japan). PTP1B inhibitory activity (%) is defined as $[1 - (ABS_{sample} - ABS_{blank})/(ABS_{control} - ABS_{blank})] \times 100$. ABS_{blank} is the absorbance of wells containing only the buffer and *p*NPP. $ABS_{control}$ is the absorbance of *p*-nitrophenol liberated by the enzyme in the assay system without a test sample, whereas ABS_{sample} is that with a test sample. The assays were performed in two duplicate experiments for all test samples. Oleanolic acid,[22] a known phosphatase inhibitor,[2] was used as a positive control.

3.12. Cytotoxicity assay against HCT-15 and Jurkat cells

HCT-15 and Jurkat cells were obtained from the Center for Biomedical Research, Institute of Developm Aging, and Cancer, Tohoku University (Miyagi, Japan). These cell lines were cultured in RPMI-1640 medi The medium was supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and μg/mL streptomycin. Exponentially growing cells, cultured in a humidified chamber at 37 °C containing 5.0% CO_2, were used for the experiments.

Cytotoxic activity was evaluated using the colorimetric MTT assay.[23] HCT-15 (1.0×10^4 cells in 100 μL) or Jurkat cells (2.0×10^4 cells in 100 μL) were added to each well of a 96-well plastic plate. A sample (1.0 μL in MeOH) was added to each well to make final concentrations from 0 to 35–45 μM, and the cells were incubated for 48 h at 37 °C. MTT (10 μL of 5.5 mg/mL stock solution) and a cell lysate solution (90 μL; 40% *N*,*N*-dimethylformamide, 20% sodium dodecyl sulfate, 2.0% CH_3COOH, and 0.030% HCl) were added to each well, and the plate was shaken thoroughly by agitation at room temperature overnight. The optical density of each well was measured at 570 nm using an MTP-500 microplate reader.

3.13. Cytotoxicity assay against Huh-7 cells

Cytotoxic activity against Huh-7 cells was assessed by the MTT assay with a modification to our previously described method.[24] Following the treatment of cells with test samples, 10 μL of MTT (5.0 mg/mL saline) was added to each well, the samples were incubated for 90 min at 37 °C and centrifuged (300 × g for 5 min), and the supernatant was aspirated off. The cells were lysed and solubilized by the addition of 100 μL of 0.040 N HCl in 2-propanol. The absorbance of each well was determined at 590 nm using an Inter-med model NJ-2300 Microplate Reader (Cosmo Bio Co., Ltd, Tokyo, Japan). Survival (%) was calculated relative to the control.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (21603012) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan to M.N. and the Foundation for Japanese Chemical Research to H.Y. We are grateful to the Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University for providing human cancer cell lines. We express our thanks to Dr. K. Suenaga of Keio University for providing the specific rotation data for terpiodiene, Dr. K. Ogawa of Z. Nakai Laboratory for identification of the marine sponge, Mr. T. Matsuki and S. Sato of Tohoku Pharmaceutical University for measuring mass spectra, and to Mr. T. Kaneko, K. Murakami, and H. Fujiwara of Tohoku Pharmaceutical University for their technical assistance.

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