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Note

Ceylonins G−I: spongian diterpenes from the marine sponge *Spongia ceylonensis*

Ahmed H. El-Desoky[1,2] · Hikaru Kato[1] · Sachiko Tsukamoto[1]

[1] Graduate School of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862-0973, Japan

[2] Division of Pharmaceutical Industries, Department of Pharmacognosy, National Research Centre, PO 12622, 33 El Bohouth St. (Former El Tahrir St.), Dokki, Giza, Egypt

Corresponding author

Sachiko Tsukamoto

sachiko@kumamoto-u.ac.jp

Abstract Three new spongian diterpenes, ceylonins G–I (**1**–**3**), were isolated from the marine sponge *Spongia ceylonensis* collected in Indonesia with five known spongian diterpenes (**4**–**8**). Only **4** inhibited USP7 with an IC_{50} value of 8.2 μM.

Keywords Marine sponge · *Spongia ceylonensis* · Spongian diterpene · USP7

Introduction

We recently reported the isolation of new nitrogenous spongian diterpenes, ceylonamides A–F (**10**–**15**) [1], and new spongian diterpenes with an ether-bridged bicyclic ring as ring D, ceylonins A–F (**16**–**21**) [2], from two specimens of the marine sponge *Spongia ceylonensis*, which inhibited the RANKL-induced formation of multinuclear osteoclasts (Fig. 1). During the isolation of **16**–**21** and spongia-13(16),14-dien-19-oic acid (**9**) [3], we isolated three new spongian diterpenes, ceylonins G–I (**1**–**3**), and five known compounds, *ent*-13-norisocopalen-15-al-18-oic acid (**4**) [4], 15-oxospongi-13-en-19-oic acid (**5**) [5], 16-oxospongi-13-en-19-oic acid (**6**) [5], and spongiabutenolides A (**8**) and B (**7**) [6]. We herein describe the structural elucidation of **1**–**3** and USP7 inhibitory activities of the spongian diterpenes isolated in our laboratory.

Results and discussion

The EtOAc-soluble fraction obtained from the EtOH extract of the sponge, which yielded ceylonins A–F (**16**–**21**), was further purified to afford three new spongian diterpenes, ceylonins G–I (**1**–**3**), along with five known spongian diterpenes (**4**–**8**).

Fig. 1

Ceylonin G (**1**) was obtained as a white amorphous solid. HRESITOFMS established its molecular formula as $C_{20}H_{26}O_4$. ^1H and ^{13}C NMR spectra (Table 1) were very similar to those of spongia-13(16),14-dien-19-oic acid (**9**) [3]. 2D NMR spectra including COSY, HSQC, and HMBC spectra revealed that a methylene at C-12 in **5** was replaced with a ketone carbon in **1** (δ_C 195.9) (Fig. 2). Hydrogens on a furan ring of **1** were

observed at δ_H 7.16 (*J*=1.3 Hz, H-15) and δ_H 7.88 (*J*=1.3 Hz, H-16). H-16 was deshielded by the ketone group at C-12 compared with **9** (δ_H 7.03 and 7.06 (H-15 and H-16)) [7].

Table 1 and Fig. 2

Ceylonins H (**2**) and I (**3**) were obtained as white amorphous solids and their molecular formulae were established as $C_{20}H_{28}O_4$ by HRESITOFMS. Their ^1H and ^{13}C NMR spectra (Table 1) were almost superimposable and showed the presence of an oxygenated methine group (**2**: δ_H 4.73 (ddd, *J*=10.3, 7.1, 1.2 Hz) and δ_C 66.8; **3**: δ_H 4.94 (br t, *J*=1.4 Hz) and δ_C 61.6). Their structures corresponded to that of **9** attached with a hydroxy group at C-12, which was supported by COSY correlations (H-9/H_2-11/H-12) and HMBC correlations (**2**: H-9/C-12, H-12/C-13, C-14, and C-16; **3**: H-9/C-12) (Fig. 2). Calculated dihedral angles suggested the orientations of the hydroxyl group at C-12 in **2** and **3** as β and α, respectively (**2**: –48° (H-11α/H-12) and –164° (H-11β/H-12); **3**: 72° (H-11α/H-12) and –43° (H-11β/H-12)) (Fig. 3). These results were supported by the magnitudes of the coupling constants of H-12 (**2**: dd, *J* = 10.3 and 7.1 Hz; **3**: br t, *J* = 1.4 Hz).

Fig. 3

In order to confirm the absolute configurations of **1**–**3**, their theoretical ECD spectra were calculated by a standard calculation procedure (Fig. 4); however, the biogenetic relationship with **9** suggested that **1**–**3** adopt 4*S*,5*R*,8*R*,9*R*,10*R*-configurations.

Experimental and calculated spectra matched well and, thus, the absolute configurations of **1**–**3** were established as shown [8].

Fig. 4

Ubiquitin-specific protease 7 (USP7) has emerged as a drug target for cancer therapy and several small molecules (HBX 19,818, HBX 41,108, and P22077) have been identified as USP7 inhibitors [9–11]. These inhibitors were discovered from libraries of synthetic compounds or developed by chemical modifications in lead compounds. In order to discover structurally interesting small molecules, we have been searching for inhibitors from our library of marine invertebrate extracts and isolated spongiacidin C [12] and petroquinones [13] as USP7 inhibitors. The biological activities of compounds **1**–**21** [14] were tested in our in-house screening, and **4** inhibited USP7 with an IC_{50} value of 8.2 μM, while the IC_{50} values of other compounds were more than 50 μM. The substructure including an aldehyde group at the C-ring in **4** may be important for this inhibition.

Experimental

General

Optical rotations were measured on a JASCO DIP-1000 polarimeter in MeOH. UV absorption was measured on a JASCO V-550 spectrophotometer in MeOH. CD spectra were measured on a JASCO J-820 spectropolarimeter in MeOH. The IR spectrum was recorded on a PerkinElmer Frontier FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer in $CDCl_3$. Chemical shifts

were referenced to the residual solvent peaks (δ_H 7.24 and δ_C 77.0 for $CDCl_3$). ESIMS spectra were measured on a Bruker impact II mass spectrometer.

Isolation

The collection and extraction processes for the marine sponge (RMNH POR 10006) were reported previously [2]. The sponge (400 g, wet weight) was extracted with EtOH. The EtOAc-soluble fraction (3.5 g) of the EtOH extract of the sponge was subjected to SiO_2 column chromatography with a stepwise gradient elution using hexane/EtOAc, EtOAc, and MeOH to yield 13 fractions (Frs. 1–13). Fr. 4 (46.9 mg), which was eluted with hexane/EtOAc (8:2), was purified by gel filtration HPLC (Asahipack GS-310P column, Asahi Chemical Industry Co., Ltd., 21.5 × 500 mm; MeOH) to yield **4** (5.2 mg). Fr. 5 (279.1 mg), eluted with hexane/EtOAc (1:1), was subjected to gel filtration HPLC (Asahipack GS-310P column; MeOH) followed by purification with C30 reversed-phase HPLC (Develosil C30-UG-5 column, Nomura Chemical Co., Ltd., 20 × 250 mm) with 70% MeOH–H_2O (0–60 min), 85% MeOH–H_2O (60–80 min), and MeOH (80–140 min) to yield **5** (16.7 mg), **6** (2.7 mg), **7** (12.4 mg), **8** (16.0 mg) [14], and a fraction containing **1**–**3** (33.7 mg). The mixture was subjected to HPLC with a diol column (Inertsil Diol, GL Sciences Inc., 20 × 250 mm; CH_2Cl_2/MeOH (30:1)) to yield **1** (0.75 mg), **2** (0.11 mg), and **3** (0.70 mg).

Ceylonin G (**1**): a white amorphous solid. $[\alpha]^{21}_D$ +77° (*c* = 0.45, MeOH). UV λ_{max} (MeOH) nm (log ε): 204 (4.37), 228 (4.15), 268 (3.67) nm. ^1H and ^{13}C NMR data, see Table 1. HRESITOFMS $[M+Na]^+$ *m/z* 353.1745 (calcd for $C_{20}H_{26}O_4Na$, 353.1723).

Ceylonin H (**2**): a white amorphous solid. $[\alpha]^{21}_{D}$ +78° (*c* = 0.085, MeOH). UV λ_{max} (MeOH) nm (log ε): 206 (4.98), 218 (4.94), 276 (3.94) nm. ^{1}H and ^{13}C NMR data, see Table 1. HRESITOFMS $[M–H]^-$ *m/z* 331.1923 (calcd for $C_{20}H_{27}O_4$, 331.1915).

Ceylonin I (**3**): a white amorphous solid. $[\alpha]^{21}_{D}$ –13° (*c* = 0.54, MeOH). UV λ_{max} (MeOH) nm (log ε): 206 (4.98), 218 (4.94), 276 (3.94) nm. ^{1}H and ^{13}C NMR data, see Table 1. HRESITOFMS $[M–H]^-$ *m/z* 331.1949 (calcd for $C_{20}H_{27}O_4$, 331.1915).

ent-13-Norisocopalen-15-al-18-oic acid (**4**): $[\alpha]^{20}_{D}$ –73° (*c* = 0.29, MeOH/CH_2Cl_2 1:1) (lit. [4] –41° (*c* = 0.029, MeOH/$CHCl_3$ 1:1)).

Conformational analyses and ECD calculations for 1–3. These experiments were performed as previously described [16].

USP7 inhibition assay

This assay was performed as previously described [12, 13].

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7. In reference 3, two hydrogens (H-15 and H-16) on the furan ring of **9** were observed at δ_H 7.03 and 7.06, whereas each chemical shift was not assigned.
8. Although the positive Cotton effect was not observed for **3**, the presence of the negative Cotton effect and biogenetic relationship with **1**, **2**, and **9** were unambiguously supported the absolute configuration.

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14. Due to the limited amount, **2** was not tested.

15. When we isolated ceylonamides A–F (**10**–**15**) from the sponge *S. ceylonensis* (RMNH POR 8676), spongian diterpenes **5**–**8** were also obtained [1].

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Table 1 ^{1}H and ^{13}C NMR[a] data for **1**−**3** in $CDCl_3$

	1		**2**		**3**	
	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)
1	39.6 CH_2	0.91 td (13.1, 4.1)	39.9 CH_2	0.89 td (13.3, 4.0)	40.0 CH_2	0.96 td (13.0, 3.7)
		1.74 br d (13.1)		1.84 br d (13.3)		1.83 m
2	18.8 CH_2	1.47 br d (14.0)	18.7 CH_2	1.46 m	18.9 CH_2	1.46 m
		1.86 dt (14.0, 4.1)		1.91 dt (14.2, 4.0)		1.91 m
3	38.2 CH_2	1.02 td (13.4, 4.1)	37.9 CH_2	1.02 td (13.5, 4.0)	38.0 CH_2	1.03 td (13.3, 4.3)
		2.17 br d (13.4)		2.16 br d (13.5)		2.15 br d (13.3)
4	43.6 C		43.6 C		43.7 C	
5	56.7 CH	1.19 dd (12.4, 2.4)	56.7 CH	1.13 m	57.2 CH	1.20 m
6	19.5 CH_2	1.94 dddd (14.4, 3.5, 3.2, 2.4)	19.9 CH_2	1.88 m	20.2 CH_2	1.89 m
		2.06 dddd (14.4, 13.0, 12.4, 3.2)		2.02 qd (13.6, 3.1)		2.02 qd (13.1, 3.2)
7	39.7 CH_2	1.61 td (13.0, 3.5)	40.7 CH_2	1.46 m	40.7 CH_2	1.54 m
		2.21 dt (13.0, 3.2)		2.14 m		2.11 dt (12.8, 2.9)
8	34.0 C		34.2 C		34.3 C	
9	54.9 CH	1.69 dd (13.3, 3.5)	54.9 CH	1.14 m	49.6 CH	1.52 m
10	28.2 C		38.2 C		37.6 C	
11	36.2 CH_2	2.46 dd (17.8, 13.3)	30.2 CH_2	1.50 m	28.6 CH_2	1.81 m
		2.53 dd (17.8, 3.5)		2.09 m		1.85 m
12	195.9 C		66.8 CH	4.73 ddd (10.3, 7.1, 1.2)	61.6 CH	4.94 br t (1.4)
13	122.5 C		124.9 C		123.8 C	
14	138.8 C		136.9 C		136.0 C	
15	135.8 C	7.16 d (1.3)	135.3 CH	7.07 d (1.2)	135.3 CH	7.08 d (1.4)
16	143.8 C	7.88 d (1.3)	138.7 CH	7.88 t (1.2)	139.7 CH	7.37 br s
17	23.9 CH_3	1.24 s	23.9 CH_3	1.29 s	25.1 CH_3	1.16 s
18	28.8 CH_3	1.24 s	28.8 CH_3	1.23 s	28.8 CH_3	1.24 s
19	183.0 C		183.0 C		181.4 C	
20	13.8 CH_3	0.89 s	14.0 CH_3	0.84 s	14.1 CH_3	0.84 s

[a] The ^{1}H NMR spectrum was recorded at 600 MHz and the ^{13}C NMR spectrum at 150 MHz.

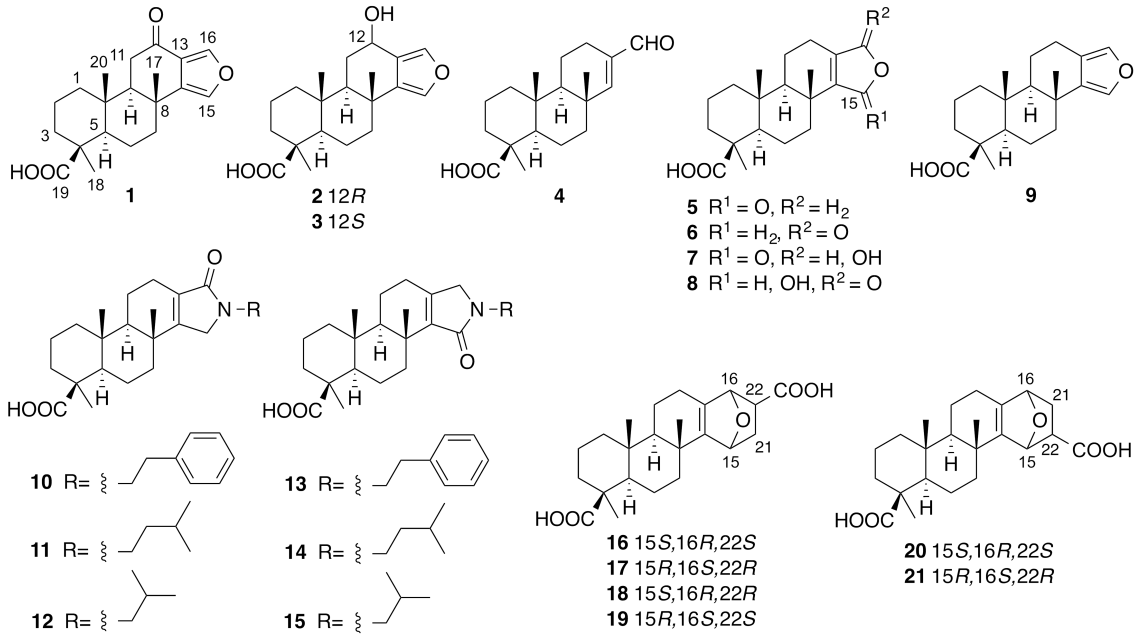

Fig. 1 Structures of **1**–**21**

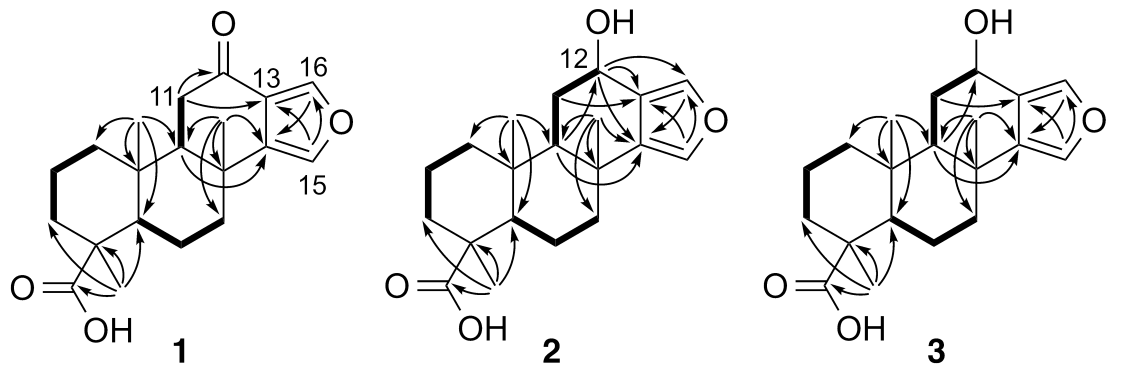

Fig. 2 COSY (bold lines) and key HMBCs (arrows) of **1**–**3**

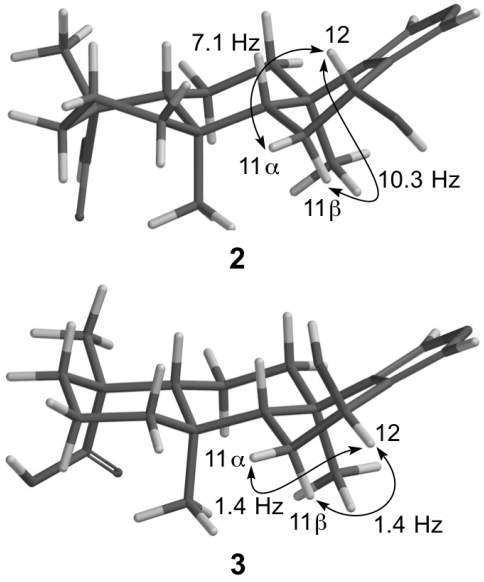

Fig. 3 Coupling constants between H_2-11 and H-12 in energy-minimized conformations of **2** and **3**

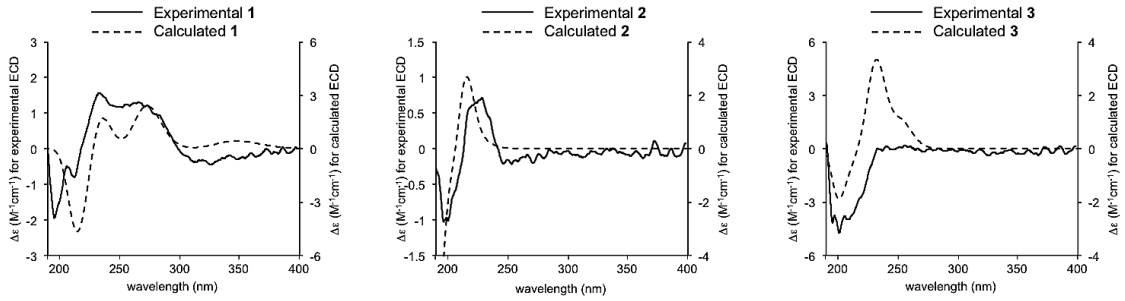

Fig. 4 Experimental ECD spectra of **1**–**3** along with the calculated spectra with 4*S*,5*R*,8*R*,9*R*,10*R*-configurations