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Simplextones A and B, Unusual Polyketides from the Marine Sponge *Plakortis simplex*

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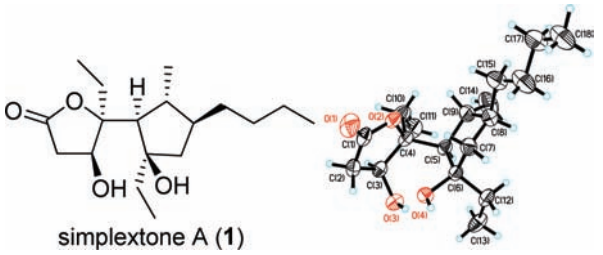
ABSTRACT



simplextone A (**1**)

Two novel polyketides, simplextones A (1) and B (2), were isolated from the sponge *Plakortis simplex*. Their structures were established by spectroscopic methods. The absolute configurations were assigned by modified Mosher's method, X-ray crystallographic analysis, and quantum mechanical calculation of the electronic circular dichroism (ECD) spectrum. Compounds 1 and 2 featured an unprecedented polyketide skeleton via the connection of a single carbon−carbon bond to form a cyclopentane. These compounds also exhibited moderate cytotoxicity.

Marine sponges of the genus *Plakortis* are well-known for their prolific production of bioactive polyketides, such as a large number of cyclic peroxides with 1,2-dioxane or 1,2-dioxolane ring systems,[1] bicyclic lactones plakortones[2] or butenolides,[3] furano α,β-unsaturated esters,[4] and fatty acid derivatives spiculoic acids.[5] Many of them exhibited significant biological activities including cytotoxic, antimalaria, and antiprotozoal activities,[6] as well as the activation of Cardiac SR-Ca^{2+}-Pumping ATPase activity.[7]

As part of our ongoing program in search of new biologically active metabolites from the South China Sea marine sponges, two novel cytotoxic metabolites, simplextones A (**1**) and B (**2**), were isolated from the methanol extract of the sponge *Plakortis simplex*. Compounds **1** and **2** featured an unprecedented butyrate-derived polyketide

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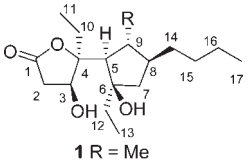
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR ($CDCl_3$) Data for Simplextones A (**1**) and B (**2**)

	simplextone A (**1**)			simplextone B (**2**)		
position	δ_H, mult (*J* in Hz)	δ_C	HMBC ($^{1}H \rightarrow ^{13}C$)	δ_H, mult (*J* in Hz)	δ_C	HMBC ($^{1}H \rightarrow ^{13}C$)
1		176.4			175.9	
2a	2.50, dd (19.0, 3.0)	39.8	1, 3, 4	2.54, dd (19.0, 2.5)	39.3	1, 3, 4
2b	2.94, dd (19.0, 9.0)		1, 3, 4	2.99, dd (19.0, 8.5)		1, 3, 4
3	4.37, dd (9.0, 3.0)	73.1	1, 10	4.34, dt (8.5, 2.5)	73.3	1, 2, 4, 10
4		95.0			94.7	
5	1.91, d (7.5)	58.3	7, 9, 10, 12, 18	2.30, dd (11.0, 8.0)	50.2	3, 4, 6, 7, 9, 10, 12
6		81.6			82.6	
7a	1.42, t (12.0)	43.9	6, 8, 12, 14	1.36, m	46.4	
7b	2.05, dd (12.0, 6.0)		5, 6, 8, 9, 12	2.11, dd (12.0, 4.5)		5, 6, 8, 9, 12, 14
8	1.25 m	44.1	18	1.62, m	36.4	
9a	2.01 m	42.1	4, 5, 8, 14, 18	2.00, m	35.0	5, 6, 7, 8
9b				1.77, m		5, 6, 7, 8
10a	1.73 m	31.6	3, 4, 5, 11	1.65, m	31.8	3, 4, 5, 11
10b	1.81 m		3, 4, 5, 11	1.79, m		3, 4, 5, 11
11	0.92, t (7.5)	7.3	4, 10	0.93, t (7.5)	7.86	4, 10
12a	1.56 m	35.5	5, 6, 7, 13	1.58, m	35.0	5, 6, 7, 13
12b	1.72 m		5, 6, 7, 13	1.83, m		5, 6, 7, 13
13	0.90, t (7.5)	7.7	6, 12	0.94, t (7.5)	8.08	6, 12
14a	1.11 m	33.8	8, 9, 15, 16	1.35, m	35.0	8, 9, 15, 16
14b	1.56 m		8, 9, 15, 16	1,58, m		8, 9, 15, 16
15a	1.21 m	30.6	8, 14, 16, 17	1.28, m	30.5	16, 17
15b	1.31 m					
16	1.31 m	22.9	14, 15, 17	1.28, m	22.9	15, 17
17	0.89, t (7.5)	14.0	15, 16	0.88, t (7.5)	14.0	15, 16
18	1.09, d (6.5)	21.5	5, 8, 9			

skeleton via the connection of a single carbon−carbon bond to form a cyclopentane. In this paper, we report the isolation, structure elucidation, plausible biogenetic pathway, and cytotoxic activity of **1** and **2**.



1 R = Me
2 R = H

The sponge *P. simplex* (B-3, 2.0 kg, dry weight) collected from the South China Sea was extracted with MeOH. The methanol residue was successively extracted with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 extract (41 g) was subjected to repeated column chromatography and purified by reversed-phase HPLC to afford compounds **1** (12.3 mg) and **2** (0.9 mg).

Simplextone A (**1**)[8] was obtained as colorless crystals. The IR absorptions indicated the presence of hydroxyl (3406 cm^{-1}) and γ-lactone carbonyl (1751 cm^{-1}) moieties. The molecular formula of **1** was established as $C_{18}H_{32}O_4$ by HRESIMS measurements ($[M + Na]^+$ *m*/*z* 335.2200) in combination with extensive NMR analysis (Table 1). The ^{1}H NMR and HSQC data revealed the presence of four aliphatic methyls (δ_H 0.89, 0.90, 0.92, and 1.09), seven methylenes, three aliphatic methines (δ_H 1.25, 1.91, and 2.01), and an oxymethine ($\delta_{H/C}$ 4.37/73.1). Additionally, the ^{13}C NMR spectrum showed the presence of one carbonyl (δ_C 176.4) and two oxygenated quaternary carbons (δ_C 81.6 and 95.0). On the basis of COSY data, these subunits were assembled into several partial structures as indicated in Figure 1: C-2/C-3, C-5/C-9/C-18, C-7/C-8, C-10/C-11, C-12/C-13, C-14/C-15, and C-16/C-17. The HMBC correlations from H_3-11 to C-4 and from H-3 to C-1 and C-10 clearly defined the partial structure C-1−C-11. The HMBC correlations from H_3-18 to C-8, from H_3-13 to C-6, from H-12b to C-5 and C-7, and from H-7b to C-5 indicated a substituted cyclopentane. The connection of the partial structure C-14/C-15 to C-16/C-17 was provided by an HMBC correlation between H_3-17 and C-15. The HMBC correlation from H-5 to C-10 confirmed the connectivity of partial structure C-1−C-11 with the cyclopentane by a bond between C-4 and C-5. The attachment of the C-14−C-17 group to C-8 was deduced from the HMBC correlations between H-9 and C-14 and between H-7a and C-14. The carbonyl of C-1 was confirmed to form a γ-butyrolactone with the oxygenated C-4 (downfield chemical shift at δ 95.0) to consume the remaining one degree of unsaturation. With this assignment secured, the final oxymethine at C-3 and the oxygenated quaternary carbons at C-6 had to be substituted with hydroxyl groups to satisfy the molecular formula. This completed the assignment of the planar structure of simplextone A (**1**) as depicted.

(8) $[\alpha]^{23}_D$ +28 (*c* 0.085, MeOH); IR (KBr) ν_{max} 3406, 2959, 2928, 1751, 1465, 1226, 1086, 968 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRESIMS *m*/*z* 335.2200 $[M + Na]^+$ (calcd for $C_{18}H_{32}O_4Na$ 335.2198).

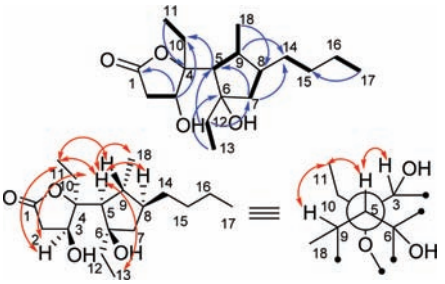


Figure 1. COSY (—), selected HMBC (blue arrow), and key NOESY (red arrow) correlations of **1**.

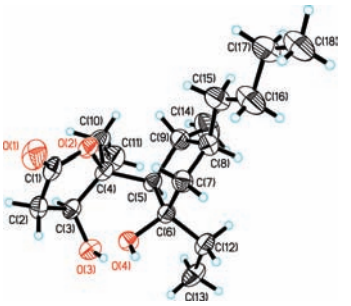


Figure 2. ORTEP diagram for simplextone A (**1**).

The relative configuration of **1** was assigned by the NOESY spectrum (Figure 1). The crucial NOE correlations between H-3 and H_3-11 suggested that they were oriented on the same side of the γ-butyrolactone moiety, while OH-3 was oriented on the opposite side. Moreover, H-5 showed NOE correlations with H-8, H_3-13, and H_3-18, suggesting that these protons were on the same face of the cyclopentane, while OH-4, H-14, and H-9 were on the opposite side. The relative configuration between the conjoined bicyclic ring systems in **1** was defined by the observation of NOESY correlations between H-3 and H-5, between H_3-11 and H-5, and between H_3-11 and H-9, which could only be accommodated by the C-4*S**/C-5*S** relative configuration shown in the Newman projection in Figure 1. These key NOESY correlations indicated that the free rotation around the C-4−C-5 bond was fixed owing to the stereohindrance of the two ethyl fragments in the molecule of **1**. The absolute configuration at C-3 was determined by applying a modified Mosher's ester method to the secondary hydroxyl group,[9,10] Compound **1** reacted with the (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chlorides to give MTPA esters **1a** and **1b**, respectively. A consistent distribution of positive and negative $\Delta\delta$ values around C-3 allowed the assignment of *S*-configuration for C-3 (Figure S1, Supporting Information). Thus, the absolute configuration of **1** was determined to be 3*S*,4*S*,5*S*,6*R*,8*S*,9*R*.

Furthermore, a successful single-crystal X-ray diffraction experiment (Figure 2) allowed the definite assignment of the relative configuration of **1**. The X-ray structure of **1** was elucidated using the differences in anomalous dispersion from Cu Kα radiation and allowed unambiguously assignment of the absolute configuration as 3*S*,4*S*,5*S*,6*R*,8*S*,9*R*. The refined Flack parameter is 0.0(2).[11] These data corroborate the modified Mosher's method of configurational assignment for the C-3 secondary alcohol.

Simplextone B (**2**)[12] was considered to have the molecular formula of $C_{17}H_{30}O_4$ based on the HRESIMS of the molecular ion peak at *m*/*z* 321.2040 $[M + Na]^+$, indicating that **2** possessed one less CH_2 unit than **1**. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except that the methine ($\delta_{H/C}$ 2.01/42.1) connected with a methyl ($\delta_{H/C}$ 1.09/21.5) in **1** was replaced by a methylene in **2**. This structure was supported by the COSY and HMBC data.

From the NOESY spectrum of **2**, H-3 was found to show an NOE correlation with H_3-11, indicating that the HO-3 and C-10/C-11 group were oriented on the opposite side of the γ-butyrolactone moiety. Furthermore, the NOE correlations were observed between H-5/H_3-13 and H-5/H-8. Thus, these protons were on the same face of the cyclopentane. On the basis of the above NOE correlations, the total set of possible candidate stereoisomers were pruned to only a subset of the four probable configurations in Figure S2 (Supporting Information).

To identify the most probable candidate for **2**, all four possible configurations with satisfactory NOE constraints were guided by comparing the actual electronic circular dichroism (ECD) trace of **2** with those predicted using time-dependent density functional theory (TD-DFT) calculations.[13] The CD spectra of simplextone B (**2**) showed a pronounced positive Cotton effect at 185 nm and negative Cotton effects at 193 and 218 nm. The computed ECD spectrum for structure 3*S*,4*S*,5*S*,6*R*,8*R* clearly reproduced both the signs and the shape of the measured ECD spectrum in Figure 3, and the computed ECD spectrum of its enantiomer 3*R*,4*R*,5*R*,6*S*,8*S* was the mirror image of that measured for **2**. Thus, the absolute configuration of **2** was established as 3*S*,4*S*,5*S*,6*R*,8*R*. The conclusion was further supported by the overlapping of the CD spectra between **1** and **2** (Figure S3, Supporting Information). It is noted that the two compounds actually fit very well, though the stereocenter at C-8 position was assigned to *S* in compound **1** but *R* in compound **2**, which is caused by the groups priorities change accorording to the sequencing rules. Figure S3 (Supporting Information) also includes the comparison of the measured and computed ECD of **1**.

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(12) Simplextone B (**2**): colorless oil; $[\alpha]_D^{23}$ +21 (*c* 0.085, CH_3OH); IR (KBr) ν_{max} 3403, 2959, 2925, 1752, 1465, 1226, 1085, 957 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRESIMS *m*/*z* 321.2040 $[M + Na]^+$ (calcd for $C_{18}H_{30}O_4Na$ 321.2042).
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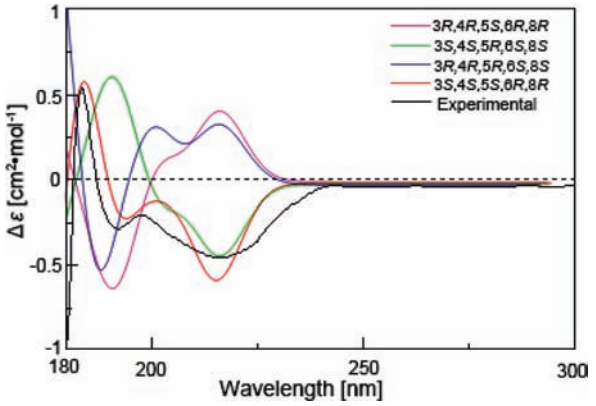
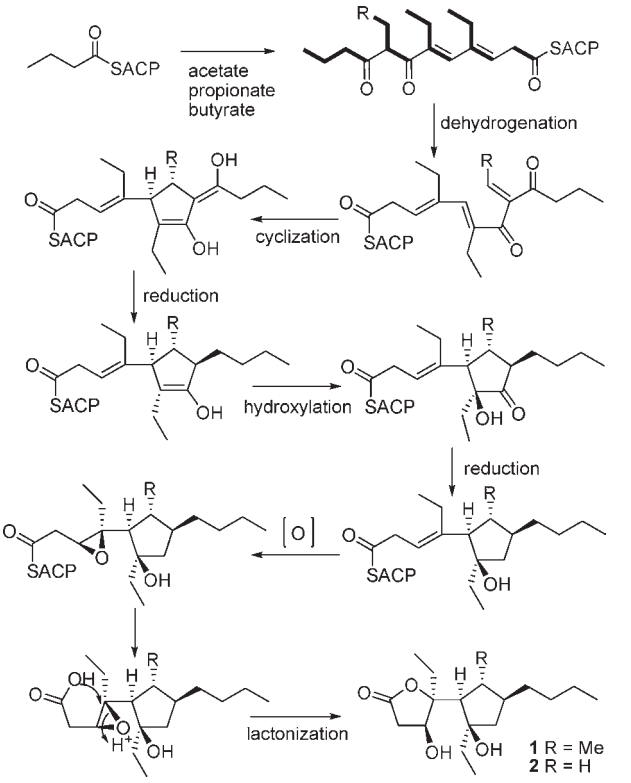


Figure 3. Experimental CD spectrum of **2** overlaid with calculated spectra for structures shown in Figure S2 (Supporting Information). The four computed ECD spectra have been normalized to the maximum negative value to ease the comparison of experimental and calculated data.

Most of the polyketides obtained from the marine sponge of genus *Plakortis* appear to involve ethyl branches[1] as a result of intact butyrate incorporation in their biosynthesis.[14,15] Simplextones A (**1**) and B (**2**) have a previously unknown polyketide skeleton via the connection of a single carbon−carbon bond to form a cyclopentane. A plausible biogenetic pathway for **1** and **2** is illustrated in Scheme 1. One acetate and four butyrate units might be required to assemble the polyketide skeleton for **1**, while **2** is assembled by one acetate, one propionate, and three butyrate units. The linear polyketide precursor further undergoes a series of reactions including dehydrogenation, cyclization, reduction, and hydroxylation to form a cyclopentane. The cyclopentane intermediate is then released from the acyl carrier protein (ACP) after being modified by the formation of an epoxide, and the opening of the epoxide intermediates leads to the insertion of a hydroxyl group with the concomitant lactonization of the γ-lactone.[16]

Simplextones A (**1**) and B (**2**) were evaluated for cytotoxicity against HCT-116 (colon cancer), SGC7901 (gastric cancer), HeLa (cervical cancer), and SW480 (colon cancer) human cancer cell lines using the

Scheme 1. Proposed Biogenesis Pathway of **1** and **2**



MTT assay.[17] Compound **1** exhibited IC_{50} values of 26.3, 57.4, 64.7, and 60.6 μM, respectively, and **2** showed IC_{50} values of 23.7, 45.8, 66.2, and 61.1 μM, respectively.

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Supporting Information Available. Experimental procedures, NMR spectra, HRESIMS spectra, IR spectra of **1** and **2**, and X-ray data of **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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