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Aplysinoplides A−C, Cytotoxic Sesterterpenes from the Marine Sponge *Aplysinopsis digitata*

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Three sesterterpenoids, aplysinoplides A−C (**1**−**3**), were isolated from the marine sponge *Aplysinopsis digitata*. Their structures were determined on the basis of spectroscopic data. They exhibited cytotoxic activity against P388 mouse leukemia cells.

Manoalide is a sesterterpenoid with an α,β-unsaturated δ-lactol moiety, which was first isolated as an antibacterial agent from the marine sponge *Luffariella variabilis*.[1] A wide variety of sesterterpenes related to manoalide have been reported from sponges of the genera *Fascaplysinopsis*,[2] *Hyrtios*,[3] *Luffariella*,[4] *Thorecta*,[5] *Thorectandra*,[6] *Fasciospongia*,[7] *Cacospongia*,[8] *Sarcotragus*,[9] and *Acanthodendrilla*.[10] Manoalide is also well known for its potent anti-inflammatory activity as the selective inhibitor of phospholipase A_2 (PLA_2).[11] In the course of our search for cytotoxic compounds from marine invertebrates, we isolated three new sesterterpenes related to manoalide together with manoalide from the marine sponge *Aplysinopsis digitata* collected at Oshima-shinsone. Their structures were elucidated on the basis of NMR and MS data. To the best of our knowledge, aplysinoplide C (**3**) is the first derivative that has the hydroxyl group at C-15 in this class of compounds. In this report, we describe the isolation, structure elucidation, and biological activities of these compounds.

The organic layer of the extract of the sponge was dried and subjected to the modified Kupchan procedure[12] to yield 60% MeOH, $CHCl_3$, and *n*-hexane layers. The $CHCl_3$ layer was separated by ODS flash chromatography and silica gel open column chromatography, and the cytotoxic fraction was purified by repetitive reversed-phase HPLC to give aplysinoplides A−C (**1**−**3**).

Aplysinoplide A (**1**) had a molecular formula of $C_{25}H_{36}O_4$, which was suggested by HRESIMS [*m*/*z* 423.24886, $(M + Na)^+$, Δ −2.27 mmu]. Analysis of the ^1H NMR data in conjunction with the HSQC spectrum revealed the presence of two aliphatic methyls, two vinylic methyls, eight methylenes, one of which was oxygenated, one acetal, and five protonated sp^2 carbons. The ^{13}C NMR spectrum further showed the presence of one carbonyl and five nonprotonated sp^2 carbons. Partial structures **a**−**d** were deduced from the COSY data. A 4-hydroxybutenolide moiety was assigned on the basis of the HMBC correlations, H-2/(C-1, C-3, and C-25) and H-25/C-1. The presence of a tetrasubstituted double bond (C-14 and C-15) juxtaposed to a quaternary carbon (C-19) substituted by two methyl groups (C-20 and C-21) was indicated by the HMBC data (Figure 1). The mode of connections of the above-mentioned six partial structures was unambiguously established by the HMBC data (Figure 1). The UV spectrum (λ_{max} 319 nm) supported the conjugation of the butenolide with a diene. The *E*-geometry of the Δ^4-double bond was assigned on the basis of a coupling constant of 15.8 Hz. The ROESY correlations between H-5 and H-8 and between H_2-24 and H-6 indicated the 6*E*-geometry; the ROESY correlation between H-9 and H_3-23, and the carbon chemical shift (δ 16.2) of C-23 showed the 10*E*-geometry (Figure 2).

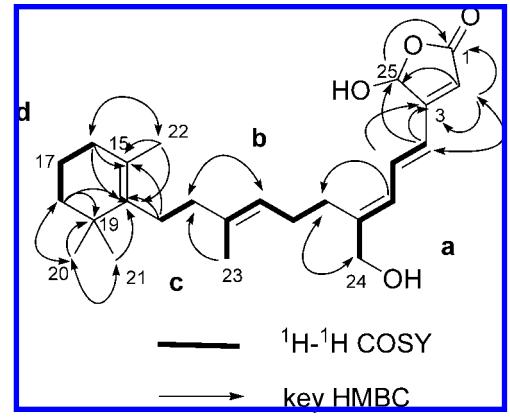
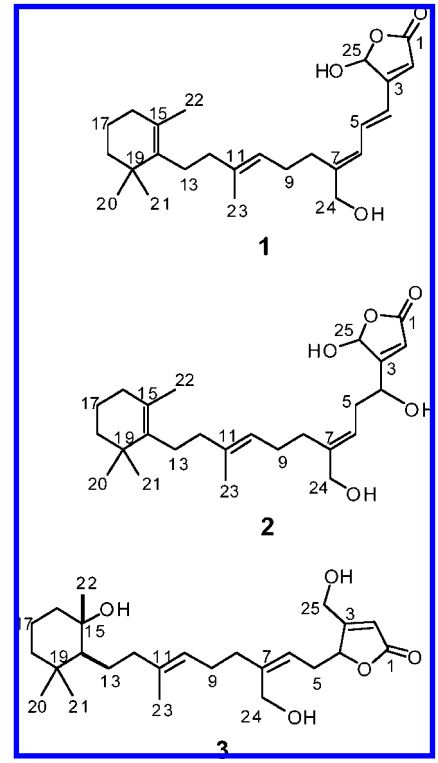


Figure 1. $^1H-^1H$ COSY and key HMBC correlations of aplysinoplide A (**1**).



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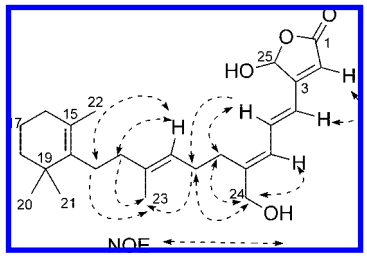

Figure 2. Selected NOE for aplysinoplide A (**1**).

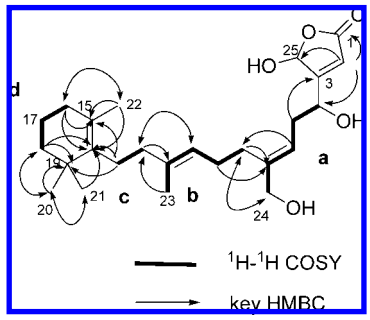

Figure 3. ^{1}H−^{1}H COSY and key HMBC correlations of aplysinoplide B (**2**).

Aplysinoplide B (**2**) had a molecular formula of $C_{25}H_{38}O_5$, which was suggested by HRESIMS [*m*/*z* 441.26188, $(M + Na)^+$, Δ +0.19 mmu]. In the ^{1}H NMR spectrum of **2**, two olefin signals at δ 6.47 and 7.30 observed for **1** were replaced by an oxymethine signal at δ 4.57. Four partial structures **a**−**d** were deduced from the COSY data (Figure 3). The presence of a 4-hydroxybutenolide and a tetrasubstituted double bond (C-14 and C-15) connected to a quaternary carbon (C-19) bearing two methyl groups was shown by the HMBC data (Figure 3). Connections of the above-mentioned partial structures were established on the basis of the HMBC data

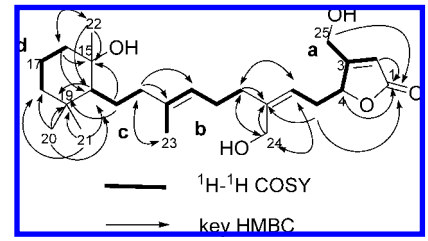

Figure 4. ^{1}H−^{1}H COSY and key HMBC correlations of aplysinoplide C (**3**).

(Figure 3). The *E*-geometry of the Δ^{10}-double bond was assigned on the basis of the ^{13}C chemical shift value of 16.2 ppm for C-23, which was supported by the NOESY correlation between H-10 and H-12. The NOESY correlations between H-5 and H-8 and between H_2-24 and H-6 indicated the 6*E*-geometry.

Aplysinoplide C (**3**) had a molecular formula of $C_{25}H_{40}O_5$, which was suggested by HRESIMS [*m*/*z* 443.27505, $(M + Na)^+$, Δ −2.29 mmu]. Partial structures **a**−**d** were deduced from the COSY data. The presence of one carbonyl carbon (δ 175.5: C-1) from the analysis of the ^{13}C NMR spectrum and the HMBC correlations (H-2 and H-4)/C-1 indicated that C-1 to C-4 in unit **a** formed a butenolide ring. Units **a** and **b** were connected via C-7, which was substituted by a hydroxymethyl group (C-24), because H_2-24 signals were coupled to C-6, C-7, and C-8 in the HMBC spectrum. Connection between units **b** and **c** was determined by an HMBC cross-peak between H_3-23 and C-12. Connection of units **c** and **d** through two quaternary carbons (C-15 and C-19) was established on the basis of the HMBC data (Figure 4). The geometry of the Δ^6-olefin was determined as *Z* on the basis of a NOESY cross-peak between H-5 and H_2−24. The *E*-geometry of the Δ^{10}-double bond was assigned on the basis of the ^{13}C chemical shift value of C-23 (δ 15.8). H-14 and OH-15 were in the *anti* relationship on the basis of the ^{13}C chemical shift values of C-14, C-15, and C-22 as compared with the literature values (*anti*-isomer δ 56.7 (C-14), 74.3 (C-15), 23.2 (C-22); *syn*-isomer δ 53.9 (C-14), 73.2 (C-15), 30.7 (C-22)).[13]

Table 1. ^{1}H and ^{13}C NMR Data for Aplysinoplides A−C (**1**−**3**) in CD_3OD

	1		**2**		**3**	
no.	δ_H, mult.	δ_C	δ_H, mult.	δ_C	δ_H, mult.	δ_C
1		173.8		173.0		175.5
2	5.95 s	116.0	6.02 s	118.1	6.00 s	115.5
3		164.5		174.2		175.0
4	6.47 (d, 15.8)	122.1	4.57 br	69.0	5.15 (t, 5.2)	83.3
5	7.30 (dd, 7.3, 15.8)	137.6	2.43 (dt, 7.2, 7.6)	34.9	2.46 (dt, 7.2, 7.6)	30.7
			2.56 br		2.80 (ddd, 4.8, 7.6, 15.2)	
6	6.30 (d, 11.6)	124.8	5.53 (t, 6.9)	121.6	5.19 (t, 7.6)	119.6
7		151.7		143.3		144.2
8	2.34 m	29.9	2.14 m	29.2	2.15 m	35.8
9	2.19 m	28.4	2.13 m	27.8	2.13 m	27.0
10	5.18 (t, 7.3)	123.8	5.18 (t, 6.6)	124.5	5.12 m	124.2
11		138.3		137.6		137.2
12	2.00 m	41.5	2.01 m	41.5	2.00 (dt, 4.6, 12.9)	43.8
					2.20 m	
13	2.04 m	29.0	2.06 m	29.0	1.32 m	26.0
					1.58 m	
14		138.2		138.2	1.13 m	57.5
15		128.2		128.1		74.5
16	1.90 (t, 5.9)	33.7	1.91 (t, 6.6)	33.7	1.38 m	43.5
					1.69 m	
17	1.58 m	20.6	1.57 m	20.6	1.51 m	20.9
18	1.41 m	41.0	1.42 m	41.0	1.20 (dt, 4.8, 12.7)	42.5
					1.34 m	
19		36.0		36.0		36.3
20	0.97 s	29.1	0.99 s	29.1	0.94 s	33.1
21	0.97 s	29.1	0.99 s	29.1	0.83 s	21.3
22	1.58 s	20.1	1.60 s	20.1	1.13 s	23.0
23	1.66 s	16.2	1.65 s	16.2	1.62 s	15.8
24	4.13 s	66.2	4.01 s	66.8	4.06 (d, 12.4)	59.5
					4.13 (d, 12.4)	
25	6.27 s	99.7	6.09 br	99.6	4.39 (d, 17.6)	58.5
					4.46 (d, 17.6)	

Aplysinoplides A−C exhibited cytotoxic activity against P388 mouse leukemia cells with IC_{50} values of 0.45, 0.45, and 11 μg/mL, respectively. Aplysinoplides A and B did not inhibit bovine pancreas PLA_2 at a concentration of 100 μM, whereas manoalide exhibited a potent activity in a parallel experiment. These results confirmed the important role of the C-24 aldehyde for inhibition of PLA_2.[14]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter in MeOH. UV spectra were measured on a Shimadzu BioSpec-1600 spectrophotometer. NMR spectra were recorded on a JEOL delta 600 NMR spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks at δ_H 3.30 and δ_C 49.0 for CD_3OD and at δ_H 2.50 and δ_C 39.5 for DMSO-d_6. ESI mass spectra were measured on a JEOL JMS-T100LC mass spectrometer.

Animal Material. The sponge *Aplysinopsis digitata* was collected by dredging at a depth of 150 m at Oshima-shinsone (28°52′ N; 129°33′ E), Kagoshima Prefecture, Japan, in 2004, immediately frozen, and kept at −20 °C until used. The voucher specimen was deposited at the Zoological Museum, University of Amsterdam (ZMAPOR 20129).

Extraction and Isolation. The sample (850 g) was extracted with MeOH (2 × 3 L) and EtOH (1 × 3 L), and the extracts were combined and concentrated *in vacuo*. The residue was suspended in H_2O (500 mL) and extracted with $CHCl_3$ (3 × 500 mL) and *n*-BuOH (2 × 500 mL). The $CHCl_3$ extract was partitioned between 90% MeOH and *n*-hexane. The 90% MeOH layer was diluted with H_2O to yield a 60% MeOH solution and then extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated and separated by ODS flash chromatography to give 12 fractions (A−L). The fractions F−H (70% and 85% MeCN fractions) were combined and separated by silica gel open column chromatography to give 10 fractions (A′−J′). The active fraction C′ ($CHCl_3$/MeOH (98:2) fraction) was further separated by reversed-phase HPLC (Cosmosil 5C_{18}-AR-II, 20 × 250 mm) with 85% MeOH followed by reversed-phase HPLC (Cosmosil 5C_{18}-AR-II, 20 × 250 mm) with 75% MeCN to give 1.7 mg of aplysinoplide A (**1**). The active fraction E′ ($CHCl_3$/MeOH (9:1) fraction) was separated by reversed-phase HPLC (Cosmosil 5C_{18}-AR-II, 20 × 250 mm) with 75% MeOH to give 10 fractions (A″−J″). The active fraction J″ was purified by reversed-phase HPLC (Cosmosil 5C_{18}-AR-II, 20 × 250 mm) with 65% MeCN to give 6.9 mg of aplysinoplide B (**2**). Fraction E″ was further separated by reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, 10 × 250 mm) with 75% MeOH followed by reversed-phase HPLC (Cosmosil 5C_{18}-MS-II, 10 × 250 mm) with 50% MeCN to give 0.8 mg of aplysonoplide C (**3**).

Aplysinoplide A (1): yellowish oil; $[\alpha]^{22.0}_D$ −10 (*c* 0.05, MeOH); UV (MeOH) 319 nm (ϵ 13 700); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 423.2489 (calcd for $C_{25}H_{36}O_4Na$, 423.2511).

Aplysinoplide B (2): yellowish oil; $[\alpha]^{22.3}_D$ +4.2 (*c* 0.3, MeOH); UV (MeOH) 203 nm (ϵ 27 500); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 441.2619 (calcd for $C_{25}H_{38}O_5Na$, 441.2617).

Aplysinoplide C (3): colorless oil; $[\alpha]^{22.7}_D$ +59.5 (*c* 0.02, MeOH); UV (MeOH) 202 nm (ϵ 28 600); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 443.2751 (calcd for $C_{25}H_{40}O_5Na$, 443.2773).

Assay for the Cytotoxicity against P388 Cells. P388 murine leukemia cells (JCRB17) were cultured in RPMI-1640 medium (Nissui Pharm. Co., Tokyo) containing 10% fetal bovine serum, 100 μg/mL of kanamycin, and 10 μg/mL of 2-hydroxyethyl disulfide at 37 °C under an atmosphere of 5% CO_2. To each well of the 96-well microplate containing 100 μL of tumor cell suspension (1 × 10^4 cells/mL) was added 100 μL of test solution dissolved in RPMI-1640 medium, and the plate was incubated in a Tabai BNA-111 CO_2 incubator (Espec Co., Tokyo, Japan) at 37 °C for 96 h. After addition of 50 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) saline solution (1 mg/mL) to each well, the plate was incubated for 3 h under the same conditions to stain live cells. After the incubation, the plate was centrifuged, the supernatants were removed, and the cells were dissolved in 150 μL of DMSO to determine the IC_{50} values.

Enzyme Assays. Inhibitory activity of aplysinoplides A and B and manoalide against PLA_2 was determined as described.[15]

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Supporting Information Available: NMR spectra for compounds **1**, **2**, and **3**. This material is available free of charge at http://pubs.acs.org.

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