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Isolation of Swinholide A and Related Glycosylated Derivatives from Two Field Collections of Marine Cyanobacteria

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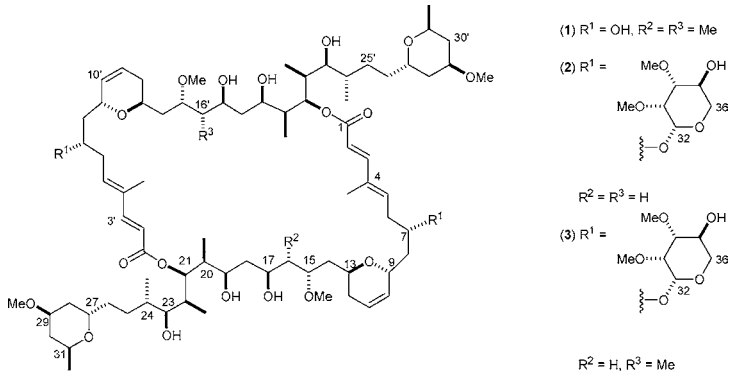
ABSTRACT



Chemical investigation of two field collections of marine cyanobacteria has led to the discovery of two new cytotoxic natural products, ankaraholides A (2) and B (3), along with the known compound swinholide A (1). Since swinholide-type compounds were previously localized to the heterotrophic bacteria of sponges, these findings raise intriguing questions about their true metabolic source.

In our ongoing search for new biologically active secondary metabolites from marine algae and cyanobacteria, we isolated swinholide A (**1**) from a Fijian cyanobacterium of the genus *Symploca* cf. sp. Bioassay-guided investigation of another cyanobacterium, *Geitlerinema* sp., from a Madagascar field collection, resulted in the isolation of two new glycosylated swinholides, ankaraholides A (**2**) and B (**3**). Swinholide A was originally isolated by Kashman and Carmely from the marine sponge *Theonella swinhoei*.[1] The structure was first described as a monomer but subsequently recognized as a dimer by Kitagawa and co-workers.[2] Later, the absolute stereostructure of swinholide A was established,[3] and the related derivatives swinholide B−H, isoswinholide A, bistheonellides A[4] and B, and a monomeric seco acid of swinholide A were isolated from marine sponges belonging to the genera *Theonella*, *Lamellomorpha*, and *Tedania*.[5] Our report here is the first isolation of this compound class from

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(4) Bistheonellides are 40-membered dilactones, whereas swinholides possess a 44-membered dilactone moiety. Bistheonellide A is also known under the name misakinolide A. Analogous to swinholide A, misakinolide A was first proposed as a monomeric macrolide and later revised to the dimeric structure and therefore renamed. See ref 5d,e.
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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds **1**−**3** in $CDCl_3$ (δ in ppm, J in Hz)

		1		**2**		**3**	
position	DEPT	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1/1′	qC	170.1		170.1		170.1	
2/2′	CH	113.3	5.79, d (15.8)	115.0	5.84, d (15.6)	115.0	5.84, d (15.3)
3/3′	CH	153.3	7.58, d (15.8)	151.9	7.51, d (15.6)	152.0	7.51, d (15.3)
4/4′	qC	134.3		134.6		134.6	
4/4′-Me	CH_3	12.3	1.81, s	12.7	1.84, s	12.7	1.84, s
5/5′	CH	142.3	6.08, dd (9.0, 5.1)	139.8	6.25, dd (6.3, 6.4)	140.2	6.26, m
6/6′	CH_2	37.4	2.17, br d (14.9)	33.5	2.45, m; 2.69, m	33.5	2.45, m; 2.69, m
			2.46, ddd (19.9, 9.7, 9.7)				
7/7′	CH	66.7	4.16, dd (7.2, 7.2)	76.7	4.07, m	76.6	4.09, m
8/8′	CH_2	41.1	1.60, m	40.1	1.35, m; 1.62, m	40.1	1.39, m; 1.62, m
9/9′	CH	65.9	4.52, br d (9.2)	69.9	4.38, m	69.9	4.38, m
10/10′	CH	129.9	5.69, d (10.2)	129.6	5.74, d (10.0)	129.9	5.78, brs
11/11′	CH	123.3	5.78, m	123.6	5.83, m	124.2	5.80, m
12/12′	CH_2	30.0	1.89, m	31.9	1.98, m; 2.00, m	31.9	1.98, m; 2.00, m
			2.28, br d (17.2)				
13/13′	CH	65.8	3.90, m	65.0	3.73, m	65.0	3.75, m
14/14′	CH_2	33.9	1.45, m; 2.15, m	39.7	1.63, m; 2.22, m	39.7	1.60, m; 2.22, m
						33.9[a]	1.23, m[a]; 1.67, m[a]
15/15′	CH	75.1	4.01, m	74.7	3.75, m	74.7; 76.0[a]	3.76, m; 3.95, m[a]
15/15′-OMe	CH_3	57.5	3.35, s	57.1	3.37, s	57.1	3.37, s
16/16′	CH	41.1	1.68, m	43.6	1.37, m; 1.65, m	43.6	1.40, m; 1.67, m
						40.5[a]	1.41, m[a]
16/16′-Me	CH_3	9.1	0.81, d (6.9)			9.7[a]	0.86, d (7.3)[a]
17/17′	CH	73.9	3.84, dd (9.5, 9.5)	69.4	4.11, m	69.3; 74.0[a]	4.12, m; 3.83, m[a]
18/18′	CH_2	38.5	1.58, m; 1.63, m	41.7	1.76, m	38.6; 35.4[a]	1.64, m; 1.63, m[a]
19/19′	CH	71.4	4.01, m	71.6	3.90, m	71.4	3.92, m
20/20′	CH	40.9	1.75, dq (9.7, 7.2)	40.7	1.69, m	41.8; 39.7[a]	1.70, m; 1.76, m[a]
20/20′-Me	CH_3	9.4	0.98, d (6.9)	9.7	0.88, d (7.0)	9.7	0.88, d (4.8); 0.92, d (5.4)[a]
21/21′	CH	74.4	5.35, d (10.8)	74.8	5.38, d (10.1)	75.3	5.39, d (10.5)
22/22′	CH	37.7	1.93, m	37.2	1.92, m	37.2	1.92, m
22/22′-Me	CH_3	9.2	0.83, d (6.9)	9.8	0.94, d (6.7)	9.8	0.94, d (7.1)
23/23′	CH	76.0	3.13, d (9.7)	76.6	3.12, d (8.5)	76.6	3.12, m
24/24′	CH	33.3	1.66, m	33.6	1.69, m	33.3	1.72, m
24/24′-Me	CH_3	17.8	0.99, d (7.2)	17.9	1.01, d (6.3)	17.9	1.01, d (5.3)
25/25′	CH_2	24.0	1.26, m; 1.38, m	24.0	1.27, m; 1.39, m	24.0	1.23, m; 1.32, m
26/26′	CH_2	29.4	1.25, m; 1.87, m	29.7	1.27, m; 1.90, m	29.7	1.29, m; 1.92, m
27/27′	CH	71.4	4.01, m	71.4	4.02, m	71.5	4.04, m
28/28′	CH_2	34.9	1.59, m; 1.82, m	35.4	1.62, m; 1.84, m	35.4	1.62, m; 1.84, m
29/29′	CH	73.3	3.54, m	73.7	3.56, m	73.4	3.57, m
29/29′-OMe	CH_3	55.3	3.33, s	55.5	3.36, s	55.7	3.36, s
30/30′	CH_2	38.7	1.17, m; 1.97, m	39.0	1.19, m; 2.01, m	38.9	1.20, m; 2.04, m
31/31′	CH	64.6	3.70, m	64.4	3.66, m	64.3	3.67, m
31/31′-Me	CH_3	21.8	1.20, d (5.9)	22.3	1.22, d (6.3)	22.0	1.23, d (6.2)
32/32′	CH			103.0	4.58, d (5.5)	103.1	4.56, d (6.1); 4.54, d (6.2)
33/33′	CH			79.6	3.30, m	79.4	3.29, m
33/33′-OMe	CH_3			58.8	3.47, s	58.8	3.47, s
34/34′	CH			84.0	3.26, m	84.0	3.26, m
34/34′-OMe	CH_3			60.0	3.61, s	60.0	3.61, s
35/35′	CH			73.0	3.38, m	73.0	3.38, m
36/36′	CH_2			62.8	3.29, m; 4.03 m	62.8	3.29, m; 4.03, m

[a] Resonances at prime atom positions

field collections of marine cyanobacteria and gives new insight into the metabolic source of these compounds in the various sponge−microorganism assemblages from which they have been reported.

The cyanobacteria samples of *Symploca* cf. sp. and *Geitlerinema* sp. were collected from the Fiji Islands and Nosy Mitso-ankaraha Island, Madagascar, respectively. Organic extracts CH_2Cl_2/MeOH (2:1) of these two cyanobacteria were potently toxic to brine shrimp. Chromatographic separation using normal-phase silica VLC and C_{18} reverse-phase SPE and HPLC, all monitored using brine shrimp toxicity, yielded swinholide A (**1**) from the Fijian sample and two new compounds, ankaraholides A (**2**) and B (**3**), from the Malagasy sample.

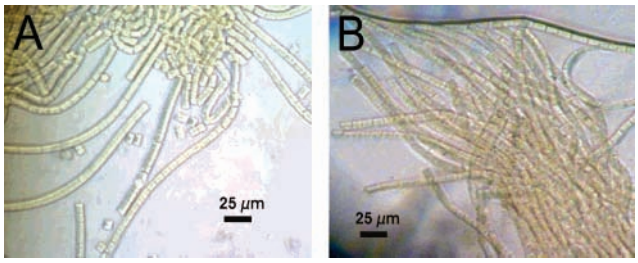

Figure 1. Photomicrographs of voucher samples of (A) *Symploca* cf. sp. from Fiji and (B) *Geitlerinema* cf. sp. from Madagascar.

The identity of swinholide A (**1**) was established by direct comparison of the ^{1}H and ^{13}C NMR, HR-MS, and $[\alpha]^{20}_{D}$ data with literature values.[1−3] The molecular formula of **2**, $C_{90}H_{152}O_{28}$, was deduced by accurate high-resolution mass measurement. Comparison of its ^{1}H and ^{13}C NMR spectral data with those for compound **1** (see Table 1) clearly showed that it was related to the swinholides. However, **2** showed only 10 upfield methyl resonances (two each at δ 0.88, 0.94, 1.01, 1.22, and 1.84), while swinholide A (**1**) possesses 12 upfield methyl groups; thus, compound **2** was a bis-normethyl derivative of swinholide A (**1**). Extensive one- and two-dimensional NMR studies of **2** revealed that the C16/C-16′ methyl groups of swinholide A (**1**) were missing. Furthermore, compound **2** showed additional resonances in the ^{13}C NMR spectrum at δ 58.8 (CH_3), 60.0 (CH_3), 62.8 (CH_2), 73.0 (CH), 79.6 (CH), 84.0 (CH), and 103.0 (CH), which suggested the presence of a bis-methoxylated sugar moiety. On the basis of vicinal $^{3}J_{H,H}$ coupling constants[6] and correlations in the ^{1}H−^{1}H COSY, HMBC, and ROESY spectral data, the sugar moiety was identified as 2,3-di-*O*-methyl-β-lyxopyranoside (Figure 2). The linkage between

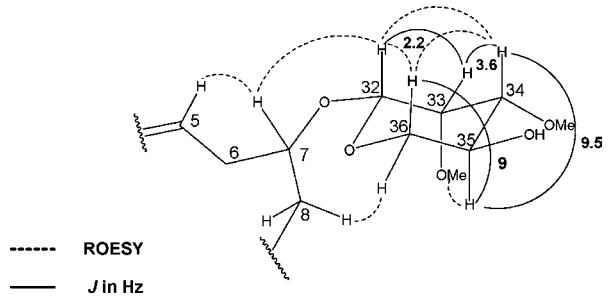

Figure 2. Coupling constant analysis and ROESY correlations of the 2,3-di-*O*-methyl-β-lyxopyranoside moiety of compound **2** ($^{3}J_{H,H}$ values were obtained in CD_3OD).[6]

the aglycon portion and the sugar unit was determined by an HMBC correlation between H-32 and C-7, as well as several ROESY correlations (see above). The relative stereochemistry of the aglycon portion was based on the close similarity of ^{1}H and ^{13}C NMR shifts and $J_{H,H}$ values between **1** and **2** and confirmed by ROESY correlations. The relative stereochemistry of the sugar and aglycon was determined by ROESY cross-peaks between H-32 and H-7 and between and H-36α and H-8α, thereby establishing the relative stereostructure of ankaraholide A (**2**) as shown.

The high-resolution MALDI-TOF mass spectrum of compound **3** showed that the molecular formula ($C_{91}H_{154}O_{28}$) was 14 mass units higher than that of ankaraholide A (**2**). In addition, the ^{1}H NMR spectrum contained a new doublet methyl resonance at δ 0.86, which showed correlations with H-16′ in the ^{1}H−^{1}H COSY spectrum and with C-15′, C-16′, and C-17′ in the HMBC spectrum. Therefore, ankaraholide B (**3**) possessed an additional methyl group at the C-16′ position relative to ankaraholide A (**2**).

Swinholide-based compounds are potent cancer cell growth inhibitors with IC_{50} values ranging from 0.37 nM to 1.0 μM against several cancer cell lines.[2,5] Swinholides exert their cytotoxic effect by disruption of the actin cytoskeleton.[7] One dimeric macrolide binds simultaneously to two molecules of G-actin, forming a tertiary complex with the two side chains of the macrolide (C-21/C-21′ to C-27/C-27′), thus inhibiting polymerization by sequestration of G-actin.[8] In contrast to the bistheonellides, the swinholides additionally cause breakage of filamentous actin strands.[9] To evaluate the influence of the sugar moieties in ankaraholide A on the biological properties of these new swinholide derivatives, **2** was tested for cytotoxicity and microfilament inhibiting activity. Ankaraholide A inhibited proliferation (IC_{50} values) in NCI−H460 (119 nM), Neuro-2a (262 nM), and MDA-MB-435 (8.9 nM) cell lines. Furthermore, in A-10 cells, **2** caused complete loss of the filamentous (F)-actin at 30 and 60 nM (Figure 3), coincident with dramatic changes in cell

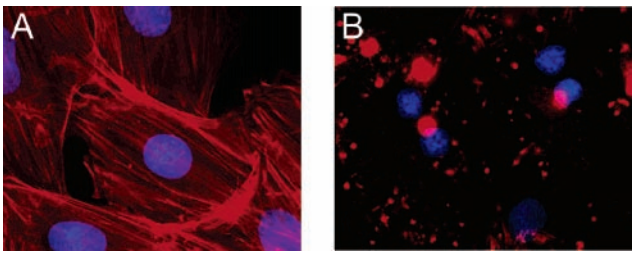

Figure 3. Effect of ankaraholide A (**2**) on the actin cytoskeleton of A-10 cells. After 24 h, cells were processed and exposed to the microfilament-staining reagent TRITC-phalloidin (visualized as red) and to the DNA-reactive compound DAPI (visualized as blue). (A) Control cells. (B) Treatment of the cells with ankaraholide A (**2**) at 60 nM, which caused complete loss of the cellular microfilament network and generated binucleated cells.

morphology. These normally fibroblastic cells became neuron-like with small core areas and multiple outwardly

(6) Proton signals of the sugar moiety were overlapped in $CDCl_3$ but resolved in CD_3OD.

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extending processes. The effects were specific for microfilaments, as there was no evidence of microtubule loss at these concentrations. Binuclear cells were present, consistent with inhibition of the actin-dependent process of cytokinesis. Hence, the mode of action as well as the biological activity of ankaraholide A (**2**) is comparable to that of swinholide A (**1**), and the additional sugar moieties in ankaraholide A do not affect its biological properties.

That the swinholides are produced by three taxonomically unrelated sponges suggests that symbiotic microorganisms may be the true producers of these metabolites. Due to the presence of cyanobacteria in these sponges and the close resemblance of swinholides to scytophycins (metabolites isolated from another cyanobacterium of the genus *Scytonema*[10]) it has been hypothesized that symbiotic cyanobacteria are the true metabolic origin of these compounds.[2,5,11] However, Bewley et al. showed that swinholide A was associated with a heterotrophic eubacterial fraction of the sponge *Theonella swinhoei*.[12] This latter approach presumes that the cellular location of a compound reflects its site of biosynthesis and neglects the possibility that substances may be excreted, diffuse within the sponge body, and absorb to cell types unrelated to its origin.

Our work reports the direct isolation of swinholides from two different cyanobacteria, thus unequivocally demonstrating that marine cyanobacteria possess the metabolic capacity to produce this skeletal class. In consequence, the true metabolic origin of the swinholides in sponges remains in question. However, it is conceivable that swinholide production occurs in multiple classes of bacteria due to gene transfer events.[13] Access to genetic methodologies and knowledge of the biosynthetic gene clusters encoding the biosynthesis of complex cyanobacterial natural products provides new techniques such as CARD-FISH analysis for probing such issues.[14]

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Supporting Information Available: Experimental details and MS and one- and two-dimensional NMR data for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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