New Steroidal Alkaloids from an Undescribed Sponge of the Genus *Corticium*

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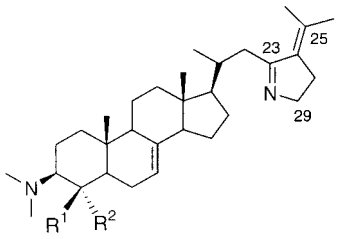
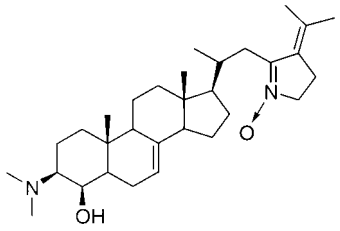
Two new steroidal alkaloids, along with a previously reported one of the plakinamine class, were isolated from the sponge *Corticium* sp. collected from Guam. The structures of the new compounds were determined by combined spectroscopic methods. These compounds exhibited moderate cytotoxicity and antifungal activity as well as DNA- and RNA-cleaving activities.

As part of our continuing search for novel secondary metabolites of biomedical and ecological importance from tropical marine animals,[1−3] we collected an undescribed sponge of the genus *Corticium* (order Homosclerophorida, family Plakinidae) from Apra Harbor, Guam. The crude extract of these specimens exhibited moderate cytotoxicity (LC_{50} 46 μg/mL) against the human leukemia cell line K562 as well as antifungal activity (diameter of clear zone 6.5 mm at 25 μg/disk in paper disk method) against *Candida albicans*. Directed by the results of bioactivity tests and ^{1}H NMR analysis, the crude extracts were separated employing solvent-partitioning followed by various chromatographic methods such as Diaion HP20 adsorption chromatography, LH-20 gel filtration chromatography, C_{18} vacuum flash chromatography, and C_{18} HPLC to afford pure secondary metabolites. Herein we report the structure elucidation of three steroidal alkaloids including two novel compounds. All of the metabolites are structurally related to plakinamine A and analogues, previously described from the sponge *Plakina* sp., by possessing an amino group at C-3 and a cyclic imine functionality on the side chain of a C_{29} steroidal carbon framework.[4−7]



1 R^1 = OH, R^2 = H

3 R^1R^2 = O



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The molecular formula of **1** was deduced as $C_{31}H_{50}N_2O$ on the basis of HREIMS and ^{13}C NMR spectrometry. By combined spectroscopic analysis, the structure of this compound was defined as lokysterolamine A previously reported as a bioactive constituent of the sponge *Corticium* sp.[5] The physical and spectral data of this compound showed good agreement with those reported previously.

Plakinamine E (**2**) was assigned the molecular formula $C_{31}H_{50}N_2O_2$ by combined HREIMS and ^{13}C NMR analysis. The spectral data of this compound were highly comparable with those of **1**. However, careful examination of the ^{13}C NMR spectra showed considerable differences between the chemical shifts of several carbons on the side chain. The most significant change occurred on the signals of the olefinic carbons at C-23−C-25. A downfield shift of over 20 ppm was observed for C-25. In the ^{1}H NMR data, differences were also found for the signals of protons located at the same positions. A combination of ^{1}H COSY, TOCSY, ROESY, gradient HSQC, and gradient HMBC experiments revealed the same ^{1}H−^{1}H and ^{1}H−^{13}C relationships throughout the entire molecule for **1** and **2**. Consequently, the spectral differences as well as the presence of an additional oxygen in the molecular formula of **2** could be accounted for by replacement of the imine with a nitrone group. This interpretation was supported by comparison of the ^{1}H NMR data of **1** and **2** with those reported for plakinamine A and its acid adduct. Chemical shifts of H-26 and H-27 were indicative of positive charge on the nitrogen: δ 2.04 and 1.83 for **1**, δ 2.27 and 2.04 for **2**, δ 1.99 and 1.79 for plakinamine A, δ 2.28 and 2.08 for the acid adduct of plakinamine A.[4] An alternative possibility of the presence of a six-membered cyclic oxime functionality (placement of an oxygen between the nitrogen and C-29 methylene) was eliminated on the basis of the upfield shift of the signal of C-29 at δ 50.3 (δ 56.1 in **1**) in the ^{13}C NMR as well as a long-range correlation between the proton signal at δ 3.95 (H-29) and the nitrogen-bearing carbon at δ 184.0 (C-23) in the HMBC data. Thus the structure of plakinamine E (**2**) was determined as the N-oxo derivative of lokysterolamine A (**1**).[5]

A closely related metabolite, plakinamine F (**3**), was isolated as a colorless gum which analyzed for $C_{31}H_{48}N_2O$ by HREIMS and ^{13}C NMR spectrometry. The ^{13}C NMR data of this compound were very similar to those obtained for **1**, with the replacement of the hydroxyl-bearing C-4 carbon at δ 67.7 by a carbonyl carbon at δ 211.6 as the most noticeable difference. The corresponding change was also observed in the ^{1}H NMR spectrum in which the H-4 oxymethine proton at δ 4.09 of **1** disappeared. In the IR

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Table 1. NMR Assignments for Compounds **2** and **3** in CD_3OD.

	2		**3**	
position	δ_H	δ_C	δ_H	δ_C
1	1.98, m	38.4 t	2.05, m; 1.70, m	37.9 t
	1.20, ddd (13.2, 13.2, 2.9)			
2	1.96, m; 1.86, br d (13.7)	20.4 t	2.16, m; 1.75, br dd (13.7, 3.4)	26.0 t
3	3.09, ddd (12.2, 3.4, 3.4)	69.4 d	3.25, dd (11.2, 5.9)	72.5 d
4	4.09, br s	67.7 d		211.6 s
5	1.36, m	46.0 d	2.37, dd (11.7, 4.4)	55.3 d
6	2.33, br dd (14.8, 14.2)	26.9 t	2.16, m; 1.88, m	22.5 t
	1.79, br d (14.8)			
7	5.30, br d (3.9)	119.0 d	5.23, br d (3.9)	117.6 d
8		139.7 s		139.5 s
9	1.71, m	51.4 d	2.02, m	50.6 d
10		35.1 s		42.6 s
11	1.56, m; 1.52, m	21.9 t	1.68, m; 1.48, m	23.2 t
12	2.07, br ddd (14.3, 3.0, 3.0)	40.5 t	2.09, ddd (13.7, 3.4, 3.4)	40.7 t
	1.32, m		1.32, m	
13		45.0 s		44.9 s
14	1.91, m	56.0 d	1.91, m	56.0 d
15	1.62, m; 1.54, m	23.9 t	1.57, m; 1.48, m	23.9 t
16	2.01, m; 1.43, m	28.7 t	2.00, m; 1.42, m	28.7 t
17	1.40, m	58.3 d	1.32, m	58.7 d
18	0.63, s	12.4 q	0.59, s	12.6 q
19	1.07, s	15.3 q	0.71, s	15.4 q
20	2.01, m	36.4 d	1.94, m	35.7 d
21	0.98, d (6.8)	19.4 q	0.89, d (6.5)	19.6 q
22	3.14, br d (14.7)	39.9 t	2.82, br d (14.7)	41.8 t
	2.61, dd (14.7, 11.2)		2.29, dd (14.7, 11.2)	
23		184.0 s		176.1 s
24		133.0 s		137.3 s
25		155.5 s		133.3 s
26	2.27, s	23.2 q	2.04, s	21.9 q
27	2.04, s	26.6 q	1.83, s	25.5 q
28	2.94, br s	29.5 t	2.59, m	32.1 t
29	3.95, t (6.8)	50.3 t	3.70, t (6.6)	56.2 t
$N-CH_3$	2.90, s	41.6 q	2.34, s	42.1 q

data, the broad absorption band at 3400 cm^{-1} of **1** was replaced by a new one at 1710 cm^{-1} in **3**. These spectral differences were readily accommodated by replacement of the C-4 carbinol by a carbonyl functionality that was confirmed by combined 2-D NMR experiments. Thus, the structure of plakinamine F (**3**) was defined as the 4-oxo derivative of lokysterolamine A (**1**).

Sponge-derived steroidal alkaloids exhibit various bioactivities.[8] For example, plakinamines A and B were reported to display antimicrobial activity,[4] while lokysterolamines A and B had cytotoxicity against various human cancer cell lines and immunomodulatory activity as well as antimicrobial activity against both bacteria and fungi.[5] Plakinamines C and D and three new analogues, recently isolated from the sponge *Corticium* sp., also exhibited cytotoxicity and anti-HIV activity.[6] In our measurements, compounds **1**−**3** also showed cytotoxicity against the human leukemia cell line K562 with LC_{50} values of 0.9, 0.2, and 1.3 μg/mL, respectively. In antifungal assays conducted by the paper disk method, compounds **1**−**3** were active against *C. albicans* with 9, 12, and 8 mm of zones of inhibition, respectively, at the concentration of 25 μg/disk. In addition, **2** totally cleaved both double-stranded DNA and 16S rRNA isolated from *E. coli* at the concentration of 10 μg/20 mL in gel electrophoresis, while **1** and **3** were inactive in the same test.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. NMR spectra were recorded in CD_3OD and $CDCl_3$ solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectral data were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. *Corticium* sp. (sample number UGI 6806) was collected by scuba at 5−15 m depth at Western Shoals, Apra Harbor, Guam, on July 17, 1996. The thickly encrusting sponge is black on the outside, lighter inside, and approximately 1 cm thick when inflated.[9] The voucher specimen (NIWA 914) is currently on deposit at the National Institute of Water & Atmospheric Research (NIWA) in Auckland, New Zealand, under the curatorship of Dr. Michelle Kelly.

Extraction and Isolation. The fresh collection was immediately frozen and kept at −25 °C until chemically investigated. The specimens were lyophilized (dry wt 41.5 g), macerated, and repeatedly extracted with MeOH (1 L × 3) and CH_2Cl_2 (1 L × 2). The combined crude extract (10.0 g) was partitioned between CH_2Cl_2 and H_2O. The H_2O layer was evaporated in vacuo, and the residue (8.15 g) was re-partitioned between *n*-BuOH (0.92 g) and H_2O (6.94 g). The *n*-BuOH layer was subjected to Diaion HP20 adsorption chromatography sequentially using H_2O, 50% aqueous MeOH, 50% aqueous acetone, MeOH, acetone, and EtOAc as eluents. The fraction eluted with 50% aqueous MeOH (346 mg) was separated by LH-20 gel filtration chromatography (*n*-hexane/CH_2Cl_2/MeOH, 4:2:1) to afford 15.7 mg of **2**. The fraction eluted with acetone (39 mg) was separated by semipreparative reversed-phase HPLC (YMC-polyamine II column, 1 cm × 25 cm, 5% aqueous EtOH) to yield 14.7 mg of **1**.

The CH_2Cl_2 layer (1.88 g) from the combined crude extract was evaporated in vacuo and re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (0.40

g) was separated by C_{18} reversed-phase vacuum flash chromatography using mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH) and finally acetone. The fraction (85 mg) eluted with 50% aqueous MeOH was dried and separated by reversed-phase HPLC (YMC-polyamine II column, 30% EtOH in MeCN) to yield 11.5 mg of pure **3** as a colorless gum.

Lokysterolamine A (**1**): colorless gum; $[\alpha]^{25}_D$ 17.7° (*c* 0.1, MeOH) [lit. 12.6° (*c* 2.0, $CHCl_3$)][3]; UV (MeOH) λ_{max} (log ϵ) 246 (3.95) nm [lit. 247 (3.91) nm][3]; IR (KBr) ν_{max} 3400 (br), 2930, 1640, 1570, 1275, 1120, 1075 cm^{-1}; HREIMS *m*/*z* 466.3935 $[M]^+$ (calcd for $C_{31}H_{50}N_2O$, 466.3923, Δ +1.2 mmu).

Plakinamine E (**2**): colorless gum; $[\alpha]^{25}_D$ 9.3° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 249 (3.85), 279 (sh, 3.56) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1640, 1455, 1375, 1270, 1120, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HMBC correlations (7 Hz) H-4/C-10; H-7/C-5, C-6, C-9, C-14; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-22/C-23; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-29/C-23, C-28; N-CH_3/C-3; HREIMS *m*/*z* 482.3867 $[M]^+$ (calcd for $C_{31}H_{50}N_2O_2$, 482.3872, Δ −0.5 mmu).

Plakinamine F (**3**): colorless gum; $[\alpha]^{25}_D$ 8.4° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.97) nm; IR (KBr) ν_{max} 2950, 2870, 1710, 1645, 1455, 1375, 1270, 1125, 1070 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 464.3772 $[M]^+$ (calcd for $C_{31}H_{48}N_2O$, 464.3767, Δ +0.5 mmu).

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