New Cytotoxic Steroidal Alkaloids from the Philippine Sponge *Corticium niger*

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Four new steroidal alkaloids, plakinamine I−K (**1**-**3**) and dihydroplakinamine K (**4**), were isolated from the marine sponge *Corticium niger*. The structures of these compounds were elucidated by interpretation of spectroscopic data. Compounds **1**−**4** exhibit significant in vitro cytotoxicity.

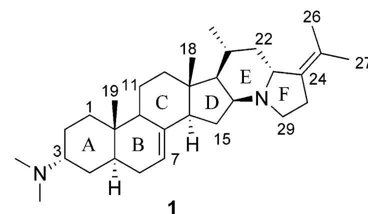
Terrestrial plants are prolific sources of steroidal alkaloids, yielding over 850 novel structures.[1] Far fewer have been reported from marine invertebrates, with only 17 reported from marine sponges.[2−7] In fact, these steroids seem to be characteristic metabolites for sponges of the genus *Corticium* (Homosclerophorida, Plakinidae), as all but two of those reported were isolated from this genus.

These compounds possess a wide range of bioactivities. For example, plakinamines A and B were reported to have antimicrobial activity,[2] while lokysterolamines A and B were found to be cytotoxic as well as having immunomodulatory and antimicrobial activities.[3] Plakinamine C and D, along with three other isolated derivatives, possessed cytotoxicity and slight anti-HIV activity.[5] In addition to cytotoxicity, plakinamines E and F exhibited antifungal and nucleic acid cleaving activities.[6] In this paper, we describe the isolation and structural elucidation of four new cytotoxic steroidal alkaloids.

Results and Discussion

As part of our continuing search for bioactive compounds, we examined a specimen of *Corticium niger* whose aqueous extract exhibited cytotoxicity in a panel of cancer cell lines. Bioassay-guided fractionation of the aqueous extract by evaluating cytotoxicity of fractions against the HCT-116 human colon tumor cell line led to the isolation of four new steroidal alkaloids, plakinamine I−K (**1**−**3**) and dihydroplakinamine K (**4**). The structures of these molecules were characterized by interpretation of NMR, IR, UV, and HRMS data.

The dihydrochloride salt of plakinamine I (**1**) was obtained as a colorless oil. The molecular formula, $C_{31}H_{50}N_2$, which was established by a high-resolution mass measurement (451.4047 [M + H], Δ +0.9 ppm), indicated eight degrees of unsaturation. An initial analysis of the ^{1}H and ^{13}C NMR spectra revealed a molecule that was steroidal in nature. A major portion of a tetracyclic backbone was assembled through interpretation of HMBC correlations from two methyl singlets at δ 0.78 and 0.93, a methyl doublet at δ 1.12, and an olefinic methine to ring junction methine carbons (Me-19 to C-5, C-9; Me-18 to C-14, C-17; Me-21 to C-17; H-7 to C-5, C-9, C-14). Additional HMBC correlations from the methyl signals established all carbons two and three bonds removed from the methyl groups. Analysis of the COSY and TOCSY data allowed assignment of the C-1/C-2/C-3/C-4/C-5/C-6/C-7, C-9/C-11/C-12, and C14/C15/C16/C17 connectivities and established the steroidal



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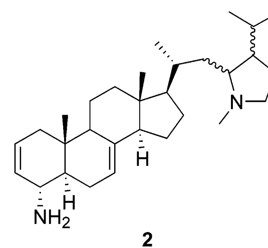

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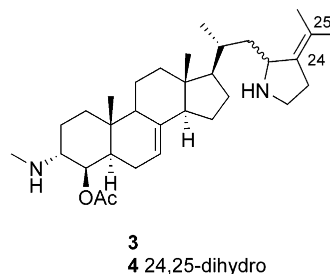

3
4 24,25-dihydro

skeleton (C-1 through C-22) with substituents required at C-3 and C-16. The chemical shift of C-3 (δ 65.3), together with an HMBC correlation from a 6H singlet at 2.92 ppm (δ_C 42.8, 42.9) to C-3, allowed placement of a dimethylamino group at this position.

In the side-chain, the 3H singlets at δ 1.73 and 1.75 were assigned to the vinyl methyl groups at C-26 and C-27, respectively, on the basis of observed HMBC correlations to the fully substituted olefinic carbons at δ 130.1 (C-24) and 129.0 (C-25). The connection of the tetrasubstituted double bond to the steroidal skeleton was established from a methine signal at δ 4.49 (H-23) which showed homoallylic coupling to Me-27 and vicinal coupling to H_2-22 in the COSY spectrum. The remaining methylene groups were assigned on the basis of homoallylic coupling from H_2-28 (δ 2.73) to Me-26 and vicinal coupling from H_2-28 to H_2-29 (δ 3.56 and 3.35). The chemical shift of C-29 (δ 53.0) and C-23 (δ 66.1) indicated nitrogen substitution and, together with an HMBC correlation observed from δ 3.56 (H-29) to C-23, established the presence of a pyrrolidine ring (ring F). Having accounted for all carbon connectivities and seven of the eight double-bond equivalents, an additional

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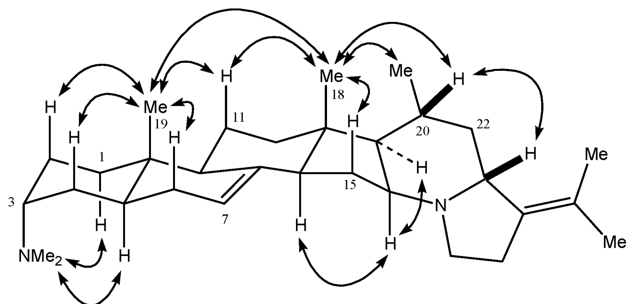

Figure 1. Selected ROESY correlations observed for plakinamine I (**1**).

ring was required. The only connection available was between the pyrrolidine nitrogen and C-16 (ring E). An HMBC correlation observed from H-29 to C-16 confirmed this assignment.

The relative stereochemistry of **1** was determined from a combination of NOE correlations observed in a ROESY experiment (Figure 1) and $^{1}H-^{1}H$ coupling constants. Observed 1,3-diaxial NOE correlations between Me-19, H-2β (δ 1.88), H_2-4 (δ 1.94), and H-6β (δ 1.80) established the chair conformation and *trans*-ring fusion of the A and B rings as well as the axial orientation of Me-19. The dimethylamino moiety was assigned as α (axial) on the basis of NOE correlations observed between the N-Me signal (δ 2.92), H-1α (δ 1.30, td, J = 14.5, 3.0), and H-5α (δ 1.60).

The *trans*-ring fusion of the C/D ring and axial orientation of Me-18 were established from NOE correlations from Me-18 to H_2-11 (δ 1.62) and H-15β (δ 1.47) observed in a ROESY experiment carried out on the free amine due to overlap of H_2-11 and H-15β in the ^{1}H NMR spectrum of the hydrochloride salt. Additional ROESY correlations from Me-18 to both Me-21 and H-20 established the β-orientation of the side-chain. The α-orientation of Me-21 and *cis*-relationship of both H-20 and H-23 were assigned from a long-range ROESY correlation observed between Me-21 and H-12β and a 1,3-diaxial NOE observed between H-20 and H-23. Finally, the β-configuration of the nitrogen substituent at C-16 was established from NOE correlations from H-16α to H-14α and H-17α. The stereochemistry of **1** was therefore assigned as 3*R**, 5*S**, 9*R**, 10*S**, 13*S**, 14*R**, 16*S**, 17*S**, 20*R**, and 23*R**.

Plakinamine J (**2**) had a molecular formula of $C_{30}H_{50}N_2$ on the basis of the HRMS and NMR spectral data. An initial analysis of the ^{1}H and ^{13}C NMR data also indicated a steroidal alkaloid similar to **1**. In the ^{1}H NMR spectrum, there were two additional olefinic signals at δ 6.05 and 5.06, which could be assigned to two carbons at 133.0 and 122.5 ppm, respectively, on the basis of an HSQC experiment. A ROESY correlation between δ 6.05 (H-2) and H_2-1 (δ 2.28 and 2.02) and another between δ 5.60 (H-3) and H-4 (δ 3.58) indicated the possibility that **2** was an unusual Δ^2 steroid. This was confirmed by observed HMBC correlations from H-1 (δ 2.28) to both C-2 and C-3 and by COSY correlations from H-2 and H-3 to H_2-1 and H-4. The ^{13}C chemical shift of C-4 (δ 53.8) required the presence of an amine substituent. A ROESY correlation between H-4 and Me-19 established the β-orientation of H-4 and allowed the placement of the amino group in the α-configuration.

In the side-chain, the two vinyl methyls present in **1** were replaced by two methyl doublets at δ_H 0.97 and 1.01, indicating that **2** had a saturated pyrrolidine ring. This was confirmed by HMBC correlations from Me-26 and Me-27 to C-24 and C-25, as well as a correlation observed from H-24 to H-23 in the COSY spectrum. The ^{1}H NMR revealed the presence of one N-Me (δ 2.93), which was placed as a substituent of the pyrrolidine ring nitrogen due to observed HMBC correlations of the N-Me to C-23 and C-29.

The relative stereochemistry of the steroidal nucleus of **2** was assigned from similar NOE correlations to those observed in **1**. ROESY correlations from H-23 to H-25, Me-26, and Me-27 established the *anti*-configuration of H-23 and H-24. Although some ROESY correlations were observed between protons on the pyrrolidine ring to Me-21, these could be rationalized for H-23 being in the α- or β-conformation. The stereochemistry of **2** is therefore defined as (4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*R**, 24*R**) or (4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*S**, 24*S**).

The molecular formula of plakinamine K (**3**), $C_{32}H_{52}N_2O_2$, suggested a steroidal alkaloid related to **1** and **2**. Analysis of the ^{1}H and ^{13}C NMR spectra revealed some significant differences. The presence of a carbonyl carbon at δ 171.6 and a methyl signal at 21.2 ppm indicated the possibility of an acetate group. This was confirmed by an HSQC correlation from a methyl proton signal at δ 2.06 to a carbon signal at δ 21.2 and an HMBC correlation from δ 2.06 to 171.6. The chemical shift of C-4 at 74.6 ppm indicated that the acetate could be placed at C-4. A ROESY correlation between Me-Ac and H-18 confirmed that placement and indicated the β-orientation of the acetate at C-4. The N-Me singlet at δ 2.39 was located on the basis of an HMBC correlation from the N-Me to the ^{13}C resonance at δ 58.8 (C-3). The α-orientation of the methylamino moiety at C-3 was established from a weak NOE between the N-Me and H-5 together with the lack of $H_{2,3}$ diaxial coupling present in H-3 (2.60, bd, J = 3 Hz), indicating the equatorial nature of this proton. COSY, HMBC, and ROESY NMR correlations allowed assignment of all other proton and carbon signals and established the stereochemistry of **3** as (3*R**, 4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*R**) or (3*R**, 4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*S**).

Comparison of ^{1}H and ^{13}C NMR spectra of 24,25-dihydroplakinamine K (**4**) revealed a steroid that contained the same steroidal backbone as **3**. The molecular formula, $C_{32}H_{54}N_2O_2$, indicated this molecule could be a dihydro derivative of plakinamine K. The presence of a saturated pyrrolidine side-chain, similar to **2**, was suggested by the presence of two methyl doublets at δ 0.93 and 1.01 (J = 6.5 Hz). The assignment of these methyl doublets as Me-26 and Me-27 was confirmed by the COSY and HMBC spectral data. The *anti*-configuration of H-23 and H-24 was established on the basis of observed ROESY correlations from H-23 to Me-26 and Me-27. Accordingly, the stereochemistry of **4** is assigned as (3*R**, 4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*R**, 24*R**) or (3*R**, 4*R**, 5*S**, 9*R**, 10*S**, 13*R**, 14*R**, 17*R**, 20*R**, 23*S**, 24*S**).

The hydrochloride salts of **1**, **2**, and **4**, along with **3**, were evaluated for cytotoxicity against the human colon tumor cell line HCT-116. Compounds **3** and **4** were most potent, both exhibiting an IC_{50} value of 1.4 μM. Compounds **1** and **2** were also active, with IC_{50} values of 10.6 and 6.1 μM, respectively. Since these compounds exhibited moderate cytotoxicity, we screened **1**, **2**, and **3** in the Bristol-Myers Squib Pharmaceutical Research Institute 11 cancer cell line panel. Compound **3** was the most potent (mean IC_{50} = 1.6 μM, max. IC_{50}/min. IC_{50} = 5), while compound **1** exhibited the greatest selectivity (mean IC_{50} = 5.6 μM, max. IC_{50}/min. IC_{50} = 25). Compound **2** also had significant cell panel results, with a mean IC_{50} = 6 μM and max. IC_{50}/min. IC_{50} = 13.

Table 1. ^{1}H, ^{13}C, and HMBC NMR Data of Compounds **1** and **2** (CD_3OD)[a]

	1			**2**		
C no.	δ_C	δ_H[b]	HMBC	δ_C	δ_H[b]	HMBC
1	32.7 CH_2	1.74 m	C-5	40.0 CH_2	2.28 dd (18, 6)	C-2, C-3, C-5, C-10
		1.30 td (14.5, 3)	C-5, C-9		2.02 m	
2	23.3 CH_2	2.12 dt (16,3)		133.0 CH	6.05 br m	
		1.88 m				
3	65.3 CH	3.31 obscured m		122.5 CH	5.60 br d (5)	
4	29.2 CH_2	1.94 m	C-5	53.8 CH	3.58 m	
5	36.2 CH	1.60 m		43.0 CH	1.71 m	C-4, C-6, C-10, C-8
6	29.8 CH_2	1.88 m	C-5, C-7, C-8	27.3 CH_2	2.35 m	C-5, C-10
		1.80 m			1.92 m	
7	119.3 CH	5.32 br d (3.5)	C-5, C-6, C-9, C-14	116.8 CH	5.25 br d (4.5)	C-5, C-9, C-14
8	138.1 C			140.3 C		
9	49.8 CH	1.98 m	C-7, C-8[c]	49.9 CH	1.92 m	
10	35.8 C			35.9 C		
11	22.0 CH_2	1.62 m		22.6 CH_2	1.62 m	
12	40.5 CH_2	2.02 m	C-9, C-11, C-13	40.6 CH_2	2.13 dt (12.5, 3)	
		1.40 m	C-11, C-13, C-14		1.35 m	
13	44.4 C			44.6 C		
14	51.9 CH	1.98 m	C-7, C-8[c]	55.9 CH	1.92 m	
15	32.0 CH_2	2.38 ddd (8,6,3)	C-13, C-14, C-16, C-17	23.9 CH_2	1.59 m	
		1.61 m	C-13, C-14, C-16			
16	59.9 CH	3.77 dt (10,8)	C-13, C-15, C-17	29.4 CH_2	2.02 m	
					1.35 m	
17	58.0 CH	1.62 m		57.7 CH	1.35 m	
18	14.4 CH_3	0.78 s	C-12, C-13, C-14, C-17	12.3 CH_3	0.62 s	C-12, C-13, C-14, C-17
19	13.0 CH_3	0.93 s	C-1, C-5, C-9, C-10	14.4 CH_3	0.92 s	C-1, C-5, C-9, C-10
20	28.6 CH	1.68 m		35.1 CH	1.54 m	
21	21.6 CH_3	1.12 d (6)	C-17, C-20, C-22	19.1 CH_3	1.08 d (6)	C-17, C-20, C-22
22	36.8 CH_2	2.23 ddd (12.5,7.5,2)	C-17, C-20, C-21, C-23	39.5 CH_2	1.81 m	C-21, C-23
		1.40 m	C-17, C-20, C-23		1.55 m	
23	66.1 CH	4.49 br t (9)	C-25	70.5 CH	3.17 m	
24	130.1 C			52.0 CH	2.02 m	C-28
25	129.0 C			30.6 CH	1.84 m	C-24, C-28
26	21.1 CH_3	1.73 br s	C-24, C-25, C-27	17.3[d] CH_3	0.97 d (6.5)	C-24, C-25, C-27
27	22.5 CH_3	1.75 br s	C-24, C-25, C-26	22.2[d] CH_3	1.01 d (6.5)	C-24, C-25, C-26
28	27.2 CH_2	2.73 m	C-25	24.6 CH_2	2.02 m	C-24
29	53.0 CH_2	3.56 dt (12,4)	C-16, C-23, C-24, C-28	56.4 CH_2	3.60 m	C-23, C-24, C-28
		3.35 obscured m	C-16, C-28		3.06 dt (11, 9)	C-28
N-Me	42.8 CH_3	2.92 s	C-3, N-Me	39.8 CH_3	2.93 s	C-23, C-29
	42.9 CH_3	2.92 s	C-3, N-Me			

[a] NMR data for the hydrochloride salts of **1** and **2**. [b] Coupling constants in Hz are given in parentheses. [c] Unclear if one or both protons are responsible for observed correlations. [d] May be interchanged.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Autopol III polarimeter. IR and UV spectra were recorded using Perkin-Elmer 1600 FTIR and Bio 20 spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer, while all 2D experiments were performed on a Varian Inova 300 MHz NMR spectrometer. High-resolution Maldi-FTMS data were obtained on a IonSpec Ultima mass spectrometer at the Scripps Research Institute, La Jolla. All solvents were redistilled prior to use.

Biological Material. The sponge *Corticium niger* Pulitzer-Finali 1996 (collection # NCI-2854) was collected using scuba at a depth of 15−30 m at Boracay Island, Philippines, in 1998 and was immediately frozen and stored at −20 °C until extraction. This sponge was identified by Mary Kay Harper. A voucher specimen has been deposited in the SIO Benthic Invertebrate Collection (# P1184).

Extraction and Purification. The sponge was lyophilized (57.5 g dry wt) and extracted with MeOH (3 × 250 mL) and, once concentrated (7.5 g), was partitioned between EtOAc and H_2O to obtain organic (1.0 g) and aqueous extracts (6.5 g). The MeOH-soluble components of the aqueous extract were then chromatographed on Sephadex LH-20 using 1:1 MeOH/H_2O followed by medium-pressure column chromatography on Supelco Diaion HP-20SS using a gradient of acetone and H_2O to yield **3** (4.0 mg, 0.007% dry weight) and two other bioactive fractions. These fractions were further purified by HPLC using gradients of MeOH and H_2O with 0.5% TFA on C_{18} to yield the TFA salts of **1**, **2**, and **4**, which were treated with $NaHCO_3$ in aqueous solution until the solution was slightly basic to yield the respective free base alkaloids. NMR data sets were obtained on the free base and hydrochloride salt of **1** (21.1 mg, 0.04% dry weight) in order to clarify overlapping signals in the ^{1}H NMR data. Since the ^{1}H NMR spectra of **2** (5.8 mg, 0.01% dry weight) and **4** (1.8 mg, 0.003% dry weight) appeared complex, we chose to convert both to their respective hydrochloride salts with the addition of a couple drops of dilute HCl.

HCT-116 Assay. The HCT-116 cells were plated in 96-well plates and incubated overnight at 37 °C in 5% CO_2/air. Compounds were added to the plate and serially diluted. Then the plate was incubated for a further 72 h. Cell viability was then assessed at the end of this period through the use of a CellTiter 96 AQ_{ueous} non-radioactive cell proliferation assay (Promega). Inhibition concentration (IC_{50}) values are interpreted from the bioreduction of MTS/PMS by living cells into a formazan product. The first step of the assay is the addition of MTS/PMS to the sample wells followed by a 3 h incubation. The quantity of the formazan product (proportional to the number of living cells) in each well was then determined using a Molecular Devices Emax microplate reader that measured the amount of 490 nm absorbance in each well, and the IC_{50} value was calculated by a SOFTMax analysis program. Etoposide (Sigma) and DMSO (solvent) were used as positive and negative controls, respectively.

Plakinamine I (1): dihydrochloride; clear oil; $[\alpha]_D$ +45.2° (c 0.32, MeOH); IR (AgCl) 2630, 1650 cm^{-1}; UV (MeOH) 202 nm (ϵ 49 800), 247 nm (ϵ 1500); ^{1}H and ^{13}C NMR data (CD_3OD), see Table 1; ^{1}H NMR, free base (CD_3OD, 400 MHz) δ

Table 2. ^{1}H, ^{13}C, and HMBC NMR Data of Compounds **3** and **4** (CD_3OD)[a]

	3			4		
C no.	δ_C	δ_H[b]	HMBC	δ_C	δ_H[b]	HMBC
1	32.9 CH_2	1.58 m	C-5	32.1 CH_2	1.81 m	
		1.39 m			1.32 m	
2	22.6 CH_2	1.88 m		20.0 CH_2	2.11 m	
					1.87 m	
3	58.8 CH	2.60 br d (3)		58.7 CH	3.31 obscured m	
4	74.6 CH	4.96 br s		70.4 CH	5.09 br s	C-10
5	40.0 CH	1.82 m		40.2 CH	1.76 m	
6	26.6 CH_2	2.01 m	C-5	26.4 CH_2	2.07 m	
		1.64 m			1.81 m	
7	118.3 CH	5.22 br d (4)	C-6, C-9, C-14	118.4 CH	5.27 br d (4)	
8	140.0 C			139.8 C		
9	51.5 CH	1.82 m		51.0 CH	1.83 m	
10	35.7 C			35.4 C		
11	22.1 CH_2	1.58 m		22.0 CH_2	1.58 m	
12	40.9 CH_2	2.20 m	C-11, C-13, C-14	40.6 CH_2	2.13 m	
		1.28 m			1.35 m	
13	44.7 C			44.7 C		
14	56.3 CH	1.85 m		56.1 CH	1.86 m	
15	24.2 CH_2	1.54 m		24.0 CH_2	1.53 m	
16	29.2 CH_2	1.90 m		28.9 CH_2	1.96 m	
17	58.1 CH	1.22 m	C-13, C-16, C-19	57.7 CH	1.30 m	
18	12.6 CH_3	0.62 s	C-12, C-13, C-14, C-17	12.4 CH_3	0.63 s	C-12, C-13, C-14, C-17
19	15.4 CH_3	1.05 s	C-1, C-5, C-9, C-10	15.1 CH_3	1.09 s	C-1, C-5, C-9, C-10
20	35.0 CH	1.56 m	C-21, C-23	35.5 CH	1.49 m	
21	18.9 CH_3	1.07 d (6.5)	C-17, C-20, C-22	18.7 CH_3	1.07 d (6)	C-17, C-20, C-22
22	40.6 CH_2	1.54 m		39.6 CH_2	1.78 m	
		1.04 obscured m			1.46 m	
23	58.0 CH	3.84 br d (11)		61.3 CH	3.31 obscured m	
24	137.0 C			50.9 CH	1.83 m	
25	122.0 C			29.6 CH	1.81 m	C-23, C-24
26	21.3 CH_3	1.66 br s	C-24, C-25, C-27	18.0[c] CH_3	0.93 d (6.5)	C-24, C-25, C-27
27	22.0 CH_3	1.66 br s	C-24, C-25, C-26	22.0[c] CH_3	1.01 d (6.5)	C-24, C-25, C-26
28	30.9 CH_2	2.36 m		25.9 CH_2	2.07 m	
29	45.2 CH_2	3.01 dt (11, 7.5)	C-24	45.4 CH_2	3.27 m	
		2.91 m	C-23, C-24, C-28			
N-Me	34.4 CH_3	2.39 s	C-3	32.5 CH_3	2.80 s	C-3
Ac	171.6 C	2.06 s	171.6	171.3 C	2.12 s	171.3
	21.2 CH_3			20.8 CH_3		

[a] NMR data for the free base of **3** and the hydrochloride salt of **4**. [b] Coupling constants in Hz are given in parentheses. [c] May be interchanged.

5.26 (br s, 1H, H-7), 4.06 (br t, 1H, $J = 9$ Hz, H-23), 3.42 (dt, 1H, $J = 10$, 8 Hz, H-16), 3.26 (dd, 1H, $J = 12$, 7 Hz, H-29), 2.98 (dt, 1H, $J = 12$, 6.5 Hz, H-29), 2.60 (m, 1H, H-28), 2.46 (dd, 1H, $J = 15$, 6, H-28), 2.27 (s, 6H, N-Me_2), 2.17 (m, 2H, H-3, H-15), 2.11 (ddd, 1H, $J = 13.5$, 7.5, 2.5 Hz, H-22), 1.95 (br dt, 1H, $J = 12.5$, 3.5, H-12), 1.89 (m, 3H, H-2, H-9, H-14), 1.74 (m, 1H, H-20), 1.72 (br s, 3H, Me-27), 1.71−1.69 (obscured m, 4H, H-1, H-5, H_2-6), 1.69 (br s, 3H, Me-26), 1.62 (m, 2H, H_2-11), 1.56 (m, 1H, H-2), 1.51−1.44 (m, 5H, H-1, H_2-4, H-15, H-17), 1.33 (td, 1H, $J = 13$, 4.5 Hz, H-12), 1.22 (q, 1H, $J = 13$ Hz, H-22), 1.05 (d, 3H, $J = 6$ Hz, Me-21), 0.88 (s, 3H, Me-19), 0.76 (s, 3H, Me-18); ^{13}C NMR, free base (CD_3OD, 100 MHz) δ 139.1 (C-8), 135.5 (C-24), 126.1 (C-25), 119.2 (C-7), 64.7 (C-23), 63.3 (C-3), 59.6 (C-17), 58.5 (C-16), 52.3 (C-14), 52.1 (C-29), 50.5 (C-9), 44.4 (C-13), 44.2 (N-Me_2), 41.1 (C-12), 38.0 (C-22), 36.3 (C-5), 36.0 (C-10), 33.8 (C-15), 33.6 (C-4), 32.0 (C-1), 30.6 (C-6), 28.8 (C-20), 28.1 (C-28), 25.5 (C-2), 22.8 (C-27), 22.2 (C-11), 22.1 (C-21), 21.0 (C-26), 14.7 (C-18), 13.4 (C-19); HRMS m/z 451.4047 [M + H], calcd for $C_{31}H_{51}N_2$, 451.4051.

Plakinamine J (2): dihydrochloride; clear oil; $[\alpha]_D$ +25° (c 0.1, MeOH); IR (AgCl) 2720, 1650 cm^{-1}; UV (MeOH) 203 nm (ϵ 56 800), 249 nm (ϵ 2030); ^{1}H and ^{13}C NMR data (CD_3OD), see Table 1; HRMS m/z 439.4047 [M + H], calcd for $C_{30}H_{50}N_2$, 439.4047.

Plakinamine K (3): clear oil; $[\alpha]_D$ +38.4° (c 0.25, MeOH); IR (AgCl) 3320, 1730 cm^{-1}; UV (MeOH) 203 nm (ϵ 59 200), 246 nm (ϵ 1200); ^{1}H and ^{13}C NMR data (CD_3OD), see Table 2; HRMS m/z 497.4107 [M + H], calcd for $C_{32}H_{52}N_2O_2$, 497.4101.

Dihydroplakinamine K (4): dihydrochloride; clear oil; $[\alpha]_D$ +5.7° (c 0.053, MeOH); IR (AgCl) 2630, 1731 cm^{-1}; UV(MeOH) 203 nm (ϵ 54 800), 247 nm (ϵ 1350); ^{1}H and ^{13}C NMR data (CD_3OD), see Table 2; HRMS m/z 499.4261 [M + H], calcd for $C_{32}H_{54}N_2O_2$, 499.4258.

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