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J. Nat. Prod., **1994**, 57 (7), 1004-1007 • DOI:
10.1021/np50109a022 • Publication Date (Web): 01 July 2004

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TWO STEROIDAL ALKALOIDS FROM A SPONGE, *CORTICIUM* SP.

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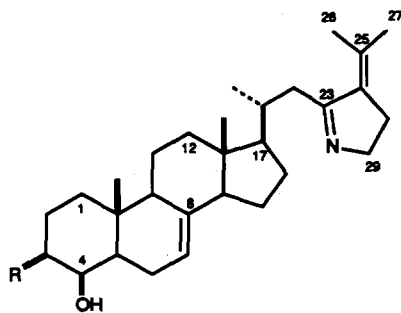
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ABSTRACT.—Lokysterolamine A and B are two steroidal alkaloids isolated from an undescribed species of the sponge genus *Corticium*, collected in Sulawesi, Indonesia. Compound A is *N,N*-dimethyl- and B is *N*-acetyl-4 β -hydroxy-3-*epi*-plakinamine A.

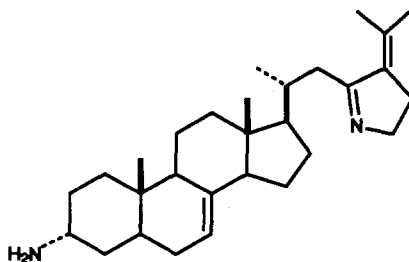
The crude extract of a sponge of the genus *Corticium*, collected in Indonesia in October 1992, showed antimicrobial activity against *Bacillus subtilis*. As a result of bioassay-guided fractionation two new steroidal alkaloids, **1** and **2**, were isolated. The compounds bear a skeletal relationship to the previously described plakinamine A [**3**] (1). In order to indicate their chemical nature, they have been called lokysterolamines.³

The hreims of the major metabolite **1**, together with its ¹³C-nmr spectrum (Table 1), indicated a molecular formula of C₃₁H₅₀N₂O. Its ¹H-nmr spectrum (Table 2) showed two methyl singlets at δ 1.04 and 0.60, and a methyl doublet at

δ 0.88, which were correlated by HMQC (proton-detected heteronuclear multiple quantum coherence) to carbons at 15.8, 12.5, and 19.5 ppm. This suggested a steroidal character of **1** with a deshielded C-19 methyl group. An ir absorption at 3400 cm⁻¹ and a carbon signal at 70.4 ppm correlated to a 3.92 ppm ¹H triplet suggested the presence of an OH group in **1**, which was subsequently located at C-4 by HMBC (proton detected heteronuclear multiple-bond correlation). Its axial conformation was compatible with the chemical shift of H-19 (δ 1.04). A small H_{3,4} coupling constant, $J=2.5$ Hz, and an nOe experiment, which showed correlations of H-4 to NMe₂, H-3, and



- 1** R=Me₂N
2 R=AcNH

**3**

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²HBOI Contribution No. 1026.

³The prefix "loky" acknowledges the important contribution of Loky Herlambang of Manado, Indonesia, to the success of the Indonesia field collection.

H-5 confirmed the stereochemistry (Figure 1).

When **1** was dissolved in CD₃OD acidified with TFA, ¹H-nmr analysis of the resulting salt confirmed that **1** bears a dimethylamino substituent. The signals of the 6H singlet and H-3 multiplet,

TABLE 1. ^{13}C -Nmr Data for **1**, **2**, and **3**
(CD_3OD , 125 MHz, ppm).

Carbon	Compound		
	1	2	3^a
1	39.6	39.2	31.8
2	21.7	23.8	29.0
3	69.2	53.7	45.6
4	70.4	72.0	35.7
5	46.6	46.2	34.5
6	27.4	27.0	29.6
7	119.4	119.1	117.7
8	139.9	139.7	139.2
9	52.0	51.8	49.6
10	35.5	35.6	34.9
11	22.0	21.9	21.2
12	40.8	40.6	39.5
13	44.8	44.7	43.6
14	56.2	56.0	55.0
15	24.0	23.9	22.8
16	28.7	28.6	27.8
17	58.8	58.6	57.1
18	12.5	12.5	12.0
19	15.8	15.7	12.0
20	35.7	35.4	34.5
21	19.5	19.5	19.3
22	41.8	41.6	31.4
23	176.2	176.1	173.2
24	137.4	137.2	137.1
25	133.3	133.2	129.0
26	21.9	21.7	21.4
27	25.4	25.5	25.4
28	32.1	32.0	41.1
29	56.3	56.1	56.1
NMe ₂	42.9		
NHAc		22.8	
		172.3	

^aData from Rosser and Faulkner (1), where the frequency was not specified.

2.34 and 2.01 ppm, were shifted downfield by 0.56 and 1.07 ppm. The lowfield shift of C-3 (69.2 ppm) suggested β -orientation of the dimethylamino group; while 3α -aminosteroids are rare, the corresponding ^{13}C -nmr chemical shift is observed at much higher field (2). This observation also was in agreement with the value of the diaxial $\text{H}_{2,3}$ coupling constant of the protonated form, $J = 12.5$ Hz, and finally was confirmed by nOe experiments, which showed a mutual correlation of H-3, but not of Me_2N , to axial H-5. The configu-

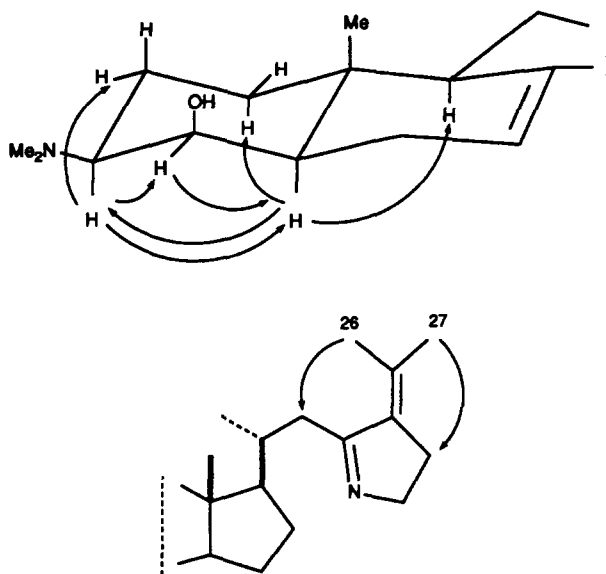
ration of the C-3 substituent in **1** is epimeric to that reported for plakinamine A **3** (1), which was established by comparison of ^{13}C -nmr shifts with synthetic 3α -amino- 5α -ergosta-7,22-diene.

In addition, the ^{13}C -nmr spectrum of **1** revealed five low-field signals. Two of them, at 119.4 and 139.9 ppm, were assigned by HMBC to a Δ^7 double bond in close analogy with plakinamine A **3**. Signals at 176.2, 137.4, and 133.3 ppm, and the uv absorption at 247 nm (ϵ 8200) were in good agreement with those reported for **3**. Comparison of the ^{13}C -nmr data (Table 1) established the nature of the side-chain and the stereochemistry at C-20. The small differences in the chemical shifts of C-23 and C-25 in **1** and **3** may be due to different sample preparation, since we noticed δ -values are somewhat concentration dependent. However, our HMQC and HMBC correlation data suggest that the C-22 and C-28 chemical shift values in plakinamine A should be interchanged. NOe correlations of H-26 to H-22 and H-27 to H-28 allowed chemical shifts assignments for the methyl groups (Figure 1). Hence lokysterolamine A **1** is *N,N*-dimethyl-4 β -hydroxy-3-*epi*-plakinamine A.

The hreims of the minor metabolite **2** together with its ^{13}C -nmr spectrum indicated a molecular formula of $\text{C}_{31}\text{H}_{48}\text{N}_2\text{O}_2$. Comparison of the spectral data with those of **1** (Tables 1 and 2), the presence of a 3H singlet at 1.94 ppm, and a low-field carbon signal at 172.3 ppm, together with a strong ir absorption at 1660 cm^{-1} , suggested an acetamido moiety at C-3 instead of dimethylamino. The ^1H -nmr signals of H-3, H-4, and H-29 in $\text{MeOH}-d_4$ overlapped. A spectrum recorded in $\text{C}_6\text{H}_6-d_6/\text{CDCl}_3$ separated them and revealed coupling constants that allowed assignment of stereochemistry in ring A. An H-4 triplet, $J = 2.5$ Hz, indicated an axial OH group, and the $\text{H}_{2,3}$ diaxial coupling constant of H-3, $J = 12.1$ Hz, suggested equatorial conformation of the acetamide. This was supported by

TABLE 2. $^1\text{H-Nmr}$ Data for **1** and **2**^{a,b}.

Proton	1	2
1	1.88, 1.04, 2H, 2×m	1.84, 1H, dt (12.5 _d , 3.1.) 1.18, 1H, dt (12.5 _t , 3.1 _d)
2	1.69, 2H, m	1.77, 1.47, 2H, 2×m
3	2.01, 1H, m	3.69, 1H, ddd (12.1, 4.2, 2.5)
4	3.92, 1H, t (2.5)	3.70, 1H, t (2.5)
5	1.24, 1H, m	1.35, 1H, ddd (14.5, 5.5, 2.5)
6	2.38, 1.74, 2H, 2×m	2.35, 1.72, 2H, 2×m
7	5.26, 1H, br s	5.25, 1H, br s
9	1.65, 1H, m	1.68, 1H, m
11	1.48, 2H, m	1.48, 2H, m
12	2.07, 1.27, 2H, m	2.05, 1H, dt (15.5 _d , 3.1.) 1.27, 1H, m
14	1.81, 1H, m	1.83, 1H, m
15	1.57, 1.45, 2H, 2×m	1.57, 1.45, 2H, 2×m
16	1.98, 1.42, 2H, 2×m	1.97, 1.42, 2H, 2×m
17	1.29, 1H, m	1.29, 1H, m
18	0.60, 3H, s	0.59, 3H, s
19	1.04, 3H, s	1.02, 3H, s
20	1.95, 1H, m	1.93, 1H, m
21	0.88, 3H, d (6.2)	0.88, 3H, d (6.2)
22	2.80, 1H, br d (16) 2.26, 1H, dd (16, 3)	2.80, 1H, br d (16) 2.27, 1H, dd (16, 3)
26	2.03, 3H, br s	2.03, 3H, br s
27	1.83, 3H, br s	1.82, 3H, br s
28	2.59, 2H, br s	2.58, 2H, br s
29	3.69, 2H, m	3.68, 2H, t (6.5)
NMe ₂	2.34, 6H, s	
NHAc		1.94, 3H, s

^aRecorded at 500 MHz in CDCl₃.^bData shown are chemical shifts in ppm and multiplicities; *J* values are in parentheses.FIGURE 1. NOe Correlations for **1**.

$n\text{Oe}$ experiments which showed the same correlations as described for **1**. In addition, the spectrum in $\text{C}_6\text{H}_6-d_6/\text{CDCl}_3$ showed an NH doublet at 5.48 ppm, coupled to H-3 ($J=8.3$ Hz).

Plakinamine A [**3**] was reported to possess antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (1). Lokysterolamine A [**1**] had in vitro activity in mouse lymphoid neoplasm (P-388), human lung carcinoma (A-549), human colon adenocarcinoma (HT-29), and human melanoma (MEL-28) assays. In addition, it showed medium immunomodulatory activity (LcV/MLR > 187), and antimicrobial and antifungal activity against *B. subtilis* and *C. albicans* (Table 3).

graphic Museum, Fort Pierce, Florida (Catalog No. 003-893).

EXTRACTION AND ISOLATION.—The sample was frozen on collection and lyophilized to yield 30 g of dry mass, which was exhaustively extracted with EtOH, followed by removal of the solvent *in vacuo*. The residue, 300 mg, was partitioned in EtOAc/H₂O, the upper layer was discarded and the lower layer extracted with *n*-BuOH. The upper layer was evaporated, after which the residue was dissolved in H₂O and rendered alkaline by addition of saturated NaHCO₃ solution. The resulting mixture was extracted with CHCl₃ followed by hplc of the organic extract on an amino column (Microsorb, Rainin) in *i*-PrOH-MeOH (9:1) and *i*-PrOH to give **1** and **2**.

Lokysterolamine A [**1**].—Colorless oil, 40 mg (0.13%); $[\alpha]_D +12.6^\circ$ ($c=2.0$, CHCl₃, 26°); hreims m/z 466.3934, M^+ , $\text{C}_{31}\text{H}_{50}\text{N}_2\text{O}$ $\Delta -1.1$ mmu; ir ν max 3400, 1645, 1575 cm^{-1} ; uv λ max 247 nm (ϵ

TABLE 3. Bioassay Data for **1** and **2**.^a

	P-388	A-549	HT-29	MEL-28	MLR	LcV	<i>B. subtilis</i>	<i>C. albicans</i>
	IC ₅₀ (μg/ml)				50 μg/disc, inhibition zone (mm)			
1	0.5	0.5	1	5	0.13	>25.0	19	11
2	1	0.5	1	>2	0.48	>12.5	8	0

^aFor definition of codes, see text.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were measured on a Perkin-Elmer 1420 spectrometer in CHCl₃. Uv spectra were determined in MeOH on a Hewlett Packard 8452A spectrophotometer. ¹H-Nmr spectra were recorded at 500 MHz and ¹³C-nmr spectra at 125 MHz on a General Electric GE W-500 spectrometer. Mass spectra were obtained with a VG 70/SE mass spectrometer. All solvents were distilled prior to use.

BIOLOGICAL MATERIAL.—The sample was collected by scuba at -20 m at Bunaken Island, Sulawesi, Indonesia, in October 1992. The sponge formed a 5-10 mm thick encrustation with a smooth mounded surface, soft liver-like texture, and was tan in life and in EtOH preservative. The sponge has a compact cortex 4-5 mm deep and is delineated by 1-2 mm wide excurrent canals. The choanosomal region is markedly less siliceous and cavernous with abundant canals. The spicules are calthrops 50-70 μm in overall diameter and candelabra are 20-40 μm in length. The sponge is an undescribed species of *Corticium* (Homosclerophorida, Plakinidae). A voucher specimen has been deposited at the Harbor Branch Ocean-

8200); ¹H and ¹³C nmr, see Tables 1 and 2.

Lokysterolamine B [**2**].—Colorless, semicrystalline solid, 6 mg (0.02%); $[\alpha]_D -3.1^\circ$ ($c=1.6$, CHCl₃, 26°); hreims m/z 480.3722, M^+ , $\text{C}_{31}\text{H}_{48}\text{N}_2\text{O}_2$ $\Delta -0.6$; ir ν max 3450, 3350, 1660 (br), 1580 cm^{-1} ; uv λ max 247 nm (ϵ 8700); ¹H and ¹³C nmr, see Tables 1 and 2.

ACKNOWLEDGMENTS

We thank Mark T. Hamann, Toshio Ichiba, and Mike Severns for collecting the sponge, and Wesley Y. Yoshida for nmr and ms assistance. J.J. thanks the University of Warsaw for sabbatical leave. We are grateful to the National Science Foundation, the Sea Grant College Program, and PharmaMar, S.A. for financial support.

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Received 3 February 1994