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LYNGBYATOXINS B AND C, TWO NEW IRRITANTS FROM *LYNGBYA MAJUSCULA*

NORIO AIMI, HIDEO ODAKA, SHIN-ICHIRO SAKAI,*

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Chiba 260, Japan

HIROTA FUJIKI,* MASAMI SUGANUMA,

National Cancer Center Research Institute, Chuo-ku, Tokyo 104, Japan

RICHARD E. MOORE,* and GREGORY M.L. PATTERSON

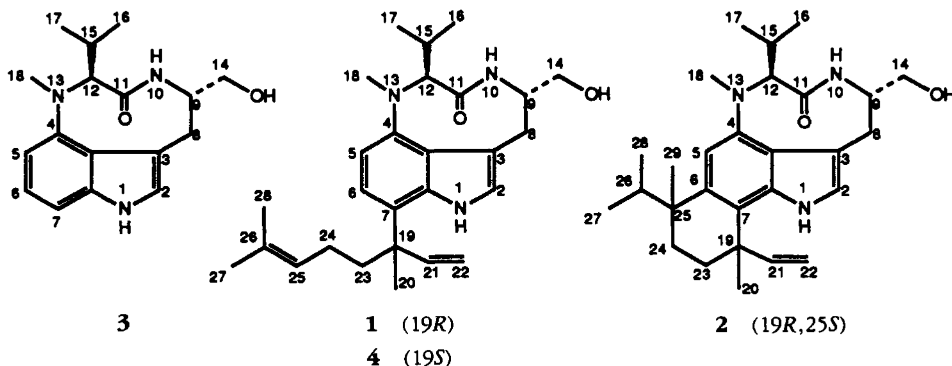
Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

ABSTRACT.—Two new irritants, lyngbyatoxins B [**5**] and C [**6**] were isolated from *Lyngbya majuscula* collected at Kahara Beach, Oahu, Hawaii, and their structures were elucidated.

Lyngbyatoxin A [**1**] was first isolated (0.02%) by Cardellina *et al.* (1) from a shallow water variety of the marine blue-green alga *Lyngbya majuscula* (Dillwyn) Harvey collected at Kahala Beach, Oahu. This compound was later found to be identical with teleocidin A-1 [**1**] (2). The structure of teleocidin A-1 was determined on the basis of spectral comparison with teleocidin B [**2**], the structure of which had been established by X-ray crystallography (3,4). The absolute configuration of **2**, and therefore of lyngbyatoxin A [**1**], was elucidated by comparison of its cd spectrum with that of indolactam V [**3**] (natural and synthetic) (5) and by chemical degradation (2).

In *Streptomyces* mycelia the metabolite teleocidin A-1 is often accompanied by the C-19 epimer, teleocidin A-2 [**4**]. However, only the former isomer appears to be present in *L. majuscula*. It is not presently known whether **1** is produced by the cyanophyte or by a microorganism associated with the alga. One of us (REM) had noted over a decade ago that minor amounts of other lyngbyatoxins were present in the alga. We were therefore prompted to reexamine *L. majuscula* from Kahala Beach to identify these other congeners and to confirm that **4** is not produced.

Extraction was carried out on the freeze-dried material by using 50% i-PrOH/CH_2Cl_2 at room temperature. Even though we could not find **4**, two new teleocidin-A-type compounds, lyngbyatoxin B [**5**] and lyngbyatoxin C [**6**], were obtained. At the same time five known compounds, malyngamides A and B, majusculamides A and B, and debromoaplysiatoxin, were isolated and characterized in addition to **1**.



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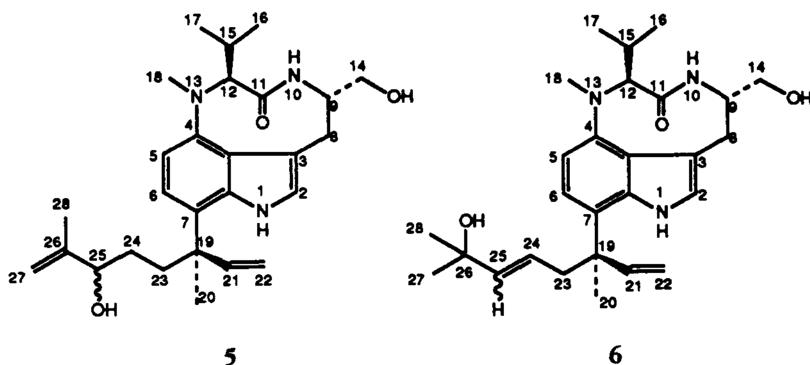
1 (19*R*)
4 (19*S*)

2 (19*R*,25*S*)



Compound **5**, an oily syrup, showed a molecular ion peak at *m/z* 453.3003 (calcd for $C_{27}H_{39}N_3O_3$, 453.2991). Its uv spectrum was superimposable on that of **1**. A second new compound, lyngbyatoxin C [**6**], had the same molecular formula, as shown by its high resolution mass spectrum (observed *m/z* 453.2987).

The ^{1}H-nmr spectrum of **5** indicated the structure depicted. [The numbering employed in this paper is based on the proposed biogenesis of the teleocidin-olivoretin class of natural products (6). For more recent results on the biosynthesis of this type of natural product, see Irie and co-workers (7,8).] It was evident that the skeletal structure consists of an indole moiety and a 9-membered lactam ring. The main difference between **5** and **1** could be seen in the ^{1}H signals for the monoterpenoidal side chain at C-7. Signals were present for terminal methylene protons on C-27 (br 1H singlets at 4.81 and 4.90 ppm), a methyl attached to a double bond at C-26 (3H singlet at 1.57 ppm), and a hydroxyl-bearing methine proton on C-25 (a broad 1H signal at 3.99 ppm which changed to a triplet with a coupling constant of 6.3 Hz on addition of D_2O) and verified the proposed structure. Further support was obtained from the eims spectrum. The molecular ion peak was the base peak, and the fragment ion arising from the cleavage between C-19 and C-23 was found at *m/z* 354 and was only moderate in intensity (24%). Other fragmentations were also quite similar to those observed for **1**.

The eims spectrum of lyngbyatoxin C [**6**] revealed a molecular ion peak at *m/z* 453, which was much smaller in intensity (8%) than the one for **5**, and a fragment peak at *m/z* 354, for cleavage between C-19 and C-23, which was the base peak. These data were compatible with the structure shown. The cleavage between C-19 and C-23 is favored because it represents allylic and benzylic cleavages. The nmr spectrum also strongly supported this structure with a 6H singlet at 1.20 ppm for the two methyl groups on C-26 and an unresolved broad singlet at 5.41–5.44 for the two olefinic protons on C-24 and C-25.

In our previous paper (2), the absolute configuration at C-19 was determined to be *R* for **1** and *S* for **4**. The cd spectra of these two compounds (Figure 1) showed the only difference at around 230 nm. This observation was further confirmed by the corresponding synthetic materials (9). The cd spectra of **5** and **6** were essentially the same as that of **1**, indicating that the configurations at C-19 are *R* for both compounds. The stereochemistry of the secondary alcohol at C-25 for **5** and the geometry of the Δ^{24} double bond for **6** remain to be determined.

The 50% inhibition for specific binding of ^{3}H-TPA ([^{3}H]12-*O*-tetradecanoylphorbol-13-acetate) were $ED_{50} = 2.2$ μM for **5** and $ED_{50} = 0.2$ μM for **6**, which correspond to 1/200 and 1/20 the activ-

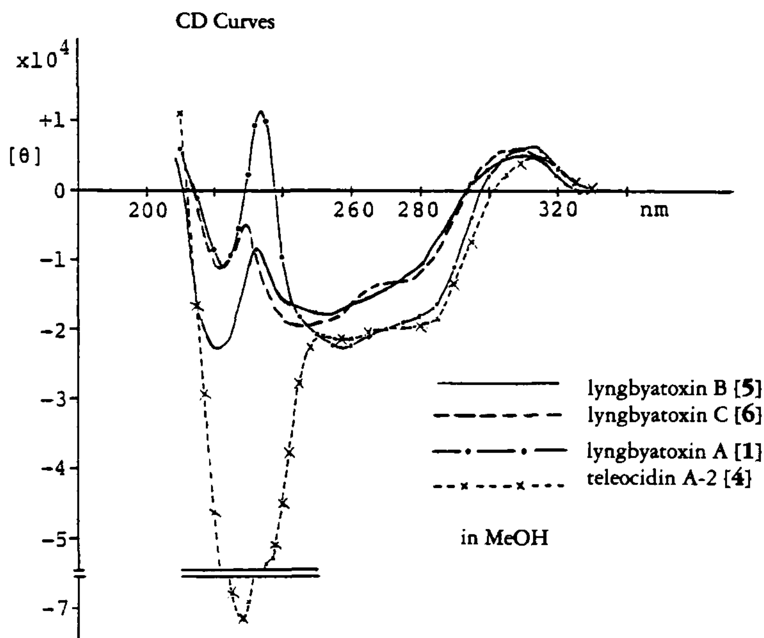


FIGURE 1. Cd spectra of lyngbyatoxins A [**1**], B [**5**], and C [**6**] and teleocidin A-2 [**4**].

ity of **1** (10). Both compounds showed a positive response in the mouse ear irritant test.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—High resolution eims data were obtained by using a Hitachi RMU 7M instrument. Eims spectra were measured by a Hitachi RMU 7M or a Hitachi RMU 60 instrument. Uv spectra were obtained using a Hitachi U-3400 spectrophotometer. JNM GSX 500 (500 MHz) and JNM GSX400 (400 MHz) instruments (JEOL) were used for ^{1}H-nmr measurements, using $CDCl_3$ as the solvent. In the ^{1}H-nmr spectra of **5** and **6**, major signals were accompanied by minor signals for conformers with relative intensities of 1:0.6. The data given below are only for the major conformers. Cd spectra were taken with a JASCO J-500A instrument. *L. majuscula* was collected at Kahala Beach, Oahu, Hawaii, and a voucher specimen has been deposited in the Department of Chemistry, University of Hawaii.

EXTRACTION AND ISOLATION.—Freeze-dried *L. majuscula* (2.68 kg) was extracted with 50% iPrOH/CH_2Cl_2 at room temperature. Fractions obtained from chromatography on an open column of SiO_2 using successively the solvent systems EtOAc-$CHCl_3$ (3:7) (300 ml) and $CHCl_3$-MeOH (97:3) (200 ml) were collected. Subsequent purification with flash cc on SiO_2 and repeated liquid chromatographic separation (normal and reversed-phase) allowed us to isolate two new compounds, lyngbyatoxin B [**5**] (0.7 mg) and lyngbyatoxin C [**6**] (1.2 mg), along with six known compounds: lyngbyatoxin A [**1**] (108 mg), malyngamide A (15 mg), malyngamide B (16 mg), majusculamide A (30 mg), majusculamide B (90 mg), and debromoaplysiatoxin (29 mg). The final purification of **5** and **6** was achieved with hplc [YMC-Pak A-024, SiO_2, 5 μ, 10 mm × 300 mm, $CHCl_3$-MeOH (97:3), 2.5 ml/min] where the retention times were 17.3 min for **5** and 18.2 min for **6**.

LYNGBYATOXIN B.—Amorphous solid: hreims *m/z* 453.3003 (calcd for $C_{27}H_{39}N_3O_3$, 453.2991); eims *m/z* (%) [M]$^+$ 453 (100), 367 (35), 354 (24), 267 (19), 223 (24); ^{1}H nmr (500 MHz) δ 0.64 (3H, d, J = 6.9 Hz, H_3-16), 0.92 (3H, d, J = 6.3 Hz, H_3-17), 1.32 (1H, m, H-24), 1.46 (3H, s, H_3-20), 1.57 (3H, s, H_3-28), 1.58 (1H, m, H-24), 1.81–1.99 (2H, m, H_2-23), 2.62 (1H, d sep, J = 10.1, 6.3 Hz, H-15), 2.91 (3H, s, H_3-18), 2.96 (1H, dd, J = 17.3, 3.6 Hz, H-8), 3.18 (1H, br d, J = 17.3 Hz, H-8), 3.52 (1H, m, H-14; +D_2O-dd, J = 10.9, 9.2 Hz), 3.74 (1H, m, H-14; +D_2O-dd, J = 10.9, 4.3 Hz), 3.99 (1H, m, H-25; +D_2O-t, J = 6.3 Hz), 4.31 (1H, m, H-9), 4.32 (1H, d,

$J = 10.2$ Hz, H-12), 4.81 (1H, s, H-27), 4.90 (1H, s, H-27), 5.27–5.32 (2H, m, H_2-22), 6.15 (1H, dd, $J = 17.6$, 11.0 Hz, H-21), 6.29 (1H, br s, N10-H), 6.49 (1H, d, $J = 8.3$ Hz, H-5), 6.82 (1H, br s, H-2), 6.98 (1H, d, $J = 8.3$ Hz, H-6), 8.48 (1H, br s, N1-H).

LYNGBYATOXIN C.—Amorphous solid: hreims *m/z* 453.2987 (calcd for $C_{27}H_{39}N_3O_3$, 453.2991); eims *m/z* (%) $[M]^+$ 453 (8), $[M - H_2O]^+$ 435 (66), 354 (100), 326 (24), 267 (40), 223 (28); ^{1}H-nmr (400 MHz) δ 0.65 (3H, d, $J = 6.8$ Hz, H_3-16), 0.93 (3H, d, $J = 6.2$ Hz, H_3-17), 1.20 (6H, s, H_3-27 and H_3-28), 1.44 (3H, s, H_3-20), 1.84 (1H, br dd, $J = 6.1$, 4.6 Hz, 14-OH), 2.51 (1H, m, H-23), 2.59 (1H, m, H-15), 2.65 (1H, m, H-23), 2.91 (3H, s, H_3-18), 2.99 (1H, m, H-8), 3.18 (1H, br d, $J = 17.8$ Hz, H-8), 3.52 (1H, m, H-14; $+D_2O$-dd, $J = 10.6$, 9.2 Hz), 3.71 (1H, m, 14-H; $+D_2O$-dd, $J = 10.6$, 4.3 Hz), 4.27 (1H, m, H-9), 4.33 (1H, d, $J = 10.4$ Hz, H-12), 5.29 (1H, dd, $J = 17.4$ Hz, H-22), 5.30 (1H, br d, $J = 11.0$ Hz, H-22), 5.41–5.44 (2H, m, H-24 and H-25), 6.19 (1H, dd, $J = 17.4$, 11.0 Hz, H-21), 6.32 (1H, br s, N10-H), 6.48 (1H, d, $J = 8.1$ Hz, H-5), 6.83 (1H, br s, H-2), 6.95 (1H, d, $J = 8.1$ Hz, H-6), 8.50 (1H, br s, N1-H).

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