# Isolation and Structure of the Cytotoxin Lyngbyabellin B and Absolute Configuration of Lyngbyapeptin A from the Marine Cyanobacterium *Lyngbya majuscula*

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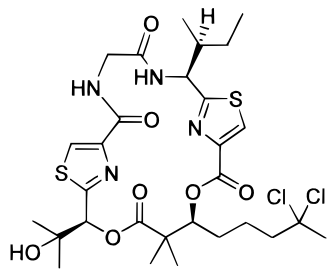
*Received March 1, 2000*

An analogue of the potent microfilament-disrupter lyngbyabellin A (**1**) has been isolated as a minor metabolite from the marine cyanobacterium *Lyngbya majuscula* collected at Apra Harbor, Guam. It possesses slightly weaker cytotoxicity than **1** and has been named lyngbyabellin B (**2**). Primarily NMR spectroscopy was used to determine its structure. The absolute configuration of **2** has been ascertained by chiral HPLC analysis of degradation products and by comparison with lyngbyabellin A (**1**). The known modified tetrapeptide lyngbyapeptin A (**3**) has also been found in the same extract, and its absolute stereochemistry could be determined for the first time.

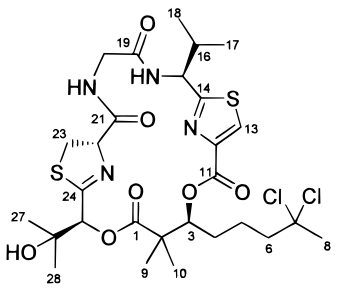
Cyanobacteria produce numerous structurally diverse secondary metabolites, including a variety of cytotoxins.[1] Extracts of several collections of a reef-inhabiting strain of *Lyngbya majuscula* Harvey ex Gomont from Guam have already yielded the cytotoxic peptolides lyngbyastatin 2,[2] norlyngbyastatin 2,[2] and lyngbyabellin A (**1**),[3] as well as the lipopeptides apramides A−G.[4] Herein, we report the isolation of lyngbyabellin B (**2**), along with the known cyanobacterial metabolite lyngbyapeptin A (**3**),[5] from the lipophilic extract of the largest re-collection of this particular cyanobacterium (UOG strain VP417).

Lyngbyabellin B (**2**) and lyngbyapeptin A (**3**) were obtained from a collection of *L. majuscula* made in January 1999, at Finger's Reef, Apra Harbor, Guam, using bioassay-guided fractionation. Both compounds were present in the same, weakly cytotoxic fraction after solvent partition and Si gel chromatography. Lyngbyabellin B (**2**) was found to be predominantly responsible for the cytotoxicity of this fraction as determined from bioassay after separation and purification by reversed-phase HPLC.

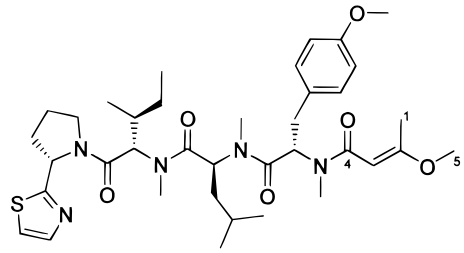
HRFABMS data indicated the molecular composition of lyngbyabellin B (**2**) as $C_{28}H_{40}Cl_2N_4O_7S_2$. The presence of two chlorine atoms was supported by the isotope peak ratio at *m*/*z* 679/681/683 of approximately 5:4:1. The molecular formula of **2** differed from the one of lyngbyabellin A (**1**) by one less carbon atom and pointed to 10 rather than 11 degrees of unsaturation. Comparison of their $^1$H and $^{13}$C NMR spectra also suggested a close relationship between **1** and **2** (Table 1). Five methyl singlets at $\delta$ 1.35 (H-10), 1.43 (H-9), 1.46 (H-27), 1.83 (H-28), and 2.08 (H-8) in the $^1$H NMR spectrum of **2** and further COSY and HMBC analysis using these signals as structural starting points for the analysis revealed the presence of 7,7-dichloro-3-acyloxy-2,2-dimethyloctanoic acid and α,β-dihydroxyisovaleric acid residues, as found in lyngbyabellin A (**1**). Doublets of doublets for methylene protons at $\delta$ 3.64 and 4.66 (H-20) were attributed to a glycine moiety ($^2J_{H,H} = -18.0$ Hz), which both **1** and **2** have in common as well. However, two methyl doublets at $\delta$ 0.77 (H-18) and 1.01 (H-17) in the $^1$H NMR spectrum of **2** instead of one doublet and one triplet in the upfield region suggested that the isoleucine-derived unit in **1** had been replaced by a valine-derived moiety in **2**. Another striking difference in their $^1$H NMR spectra was the presence of only one aromatic signal ($\delta_{H-13}$ 8.30, s), thereby indicating that merely one disubstituted thiazole ring was present. One thiazole unit was obviously replaced by a thiazoline ring, which would account for the one degree less of unsaturation for lyngbyabellin B (**2**). This finding



**1**



**2**



**3**

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10.1021/np000104n CCC: $19.00 
Published on Web 08/18/2000

**Table 1.** NMR Spectral Data for Lyngbyabellin B (**2**) in $CDCl_3$

| C/H no. | $\delta_H$[a] (*J* in Hz) | $\delta_C$[b] | HMBC[a,c,d] |
|---|---|---|---|
| 1 | | 172.6, s | H-9, H-10, H-25 |
| 2 | | 47.2, s | H-9, H-10 |
| 3 | 5.34, dd (10.9, 3.2) | 78.5, d | H-9, H-10 |
| 4 | 1.72, m, 1.95, m | 29.2, t | |
| 5 | 1.72, m | 21.6, t | |
| 6 | 2.09, m, 2.25, m | 48.8, t | H-8 |
| 7 | | 90.0, s | H-8 |
| 8 | 2.08, s | 37.4, q | |
| 9 | 1.43, s | 24.5, q | H-10 |
| 10 | 1.35, s | 22.9, q | H-9 |
| 11 | | 161.1, s | H-3,[e] H-13[e] |
| 12 | | 146.8, s | H-13 |
| 13 | 8.30, s | 129.5, d | |
| 14 | | 171.0, s | H-13, H-15 |
| 15 | 5.12, dd (9.8, 9.5) | 55.5, d | H-17, H-18 |
| 15-NH | 8.10, d (9.5) | | |
| 16 | 2.38, m | 33.9, d | H-15, H-17, H-18 |
| 17 | 1.01, d (6.7) | 19.4, q | H-18 |
| 18 | 0.77, d (6.7) | 19.7, q | H-15, H-17 |
| 19 | | 167.7, s | H-15, H-20 |
| 20 | 3.64, dd (−18.0, 2.6), 4.66, dd (−18.0, 10.0) | 43.1, t | |
| 20-NH | 6.70, dd (10.0, 2.6) | | |
| 21 | | 170.9, s | H-20, H-22, H-23 |
| 22 | 5.29, br d (9.8) | 78.73, d | |
| 23 | 3.29, dd (−11.6, 9.8), 3.76, dd (−11.6, 1.5) | 34.4, t | |
| 24 | | 177.3, s | H-22, H-23, H-25 |
| 25 | 5.74, d (1.5) | 78.67, d | H-27, H-28 |
| 26 | | 74.2, s | H-27, H-28 |
| 26-OH | 5.56, s | | |
| 27 | 1.46, s | 29.4, q | H-28 |
| 28 | 1.83, s | 26.1, q | H-27 |

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Protons showing long-range correlation with indicated carbon. [d] If not indicated otherwise, correlations were observed after optimization for $^nJ_{CH} = 7$ Hz. [e] Correlation observed after optimization for $^nJ_{CH} = 4$ Hz.

was in agreement with additional signals for a vicinal (according to the COSY spectrum) methine proton ($\delta_{H-22}$ 5.29) and methylene protons ($\delta_{H-23}$ 3.29 and 3.76). HMBC correlations of these protons to C-21 ($\delta$ 170.9) and C-24 ($\delta$ 177.3) allowed the placement of the thiazoline ring between the glycine residue and the α,*β*-dihydroxyisovaleric acid rather than adjacent to the valine-derived unit. COSY cross-peaks between H-22 and H-25 resulting from long-range coupling confirmed the connectivity, leading to the gross structure as shown for **2**.

Due to the small amount of material, only the absolute stereochemistry of the two units that are not present in **1** was established experimentally. The configuration of these stereocenters in **2** could be deduced after chiral HPLC analysis of degradation products. Compound **2** was ozonized (−78 °C, $CH_2Cl_2$) and subsequently subjected to acid hydrolysis (6 N HCl at 110 °C, 12 h). The HPLC trace of the hydrolyzate revealed that the chiral amino acid units were L-valine and D-cysteine derived (detection of the oxidation product, cysteic acid, D/L ratio 3:1),[6] allowing the assignment of the stereocenters at C-15 and C-22 as 15*S*,-22*S*. Whether the stereocenters in the 7,7-dichloro-3-acyloxy-2,2-dimethyloctanoic acid and α,*β*-dihydroxyisovaleric acid residues both possess *S* configuration as found in compound **1** has not been confirmed, but is strongly suggested by the similarity of **1** and **2**.

Lyngbyabellin B (**2**) is slightly less cytotoxic in vitro than lyngbyabellin A (**1**). Compound **2** exhibits $IC_{50}$ values of 0.10 and 0.83 μg/mL against KB and LoVo cells, respectively. It was demonstrated earlier that **1** is a potent microfilament-disrupting agent.[3] The same mode of action is expected for **2**.

Lyngbyapeptin A (**3**) was the major compound in the lyngbyabellin B-containing fraction after Si gel chromatography, but did not contribute significantly to the cytotoxicity of this fraction ($IC_{50} > 5$ μg/mL against KB and LoVo). Compound **3** was originally isolated by Klein et al.[5] from a collection of the marine cyanobacterium *Lyngbya bouillonii* Hoffmann et Demoulin[7] taken from the coast of Papua New Guinea. This modified tetrapeptide was unstable in the NMR solvent $CDCl_3$ and decomposed, obstructing the elucidation of its stereochemistry and preventing further physical characterization and testing for biological activity. In our case, peptide **3** survived complete analysis.[8] $^1H$ and $^{13}C$ NMR data for our **3** corresponded essentially to the reported data for **3**, indicating the same relative stereochemistry. Klein et al. had only suspected the *E* configuration of the double bond of the 3-methoxy-2-butenoic acid unit.[5] We could unambiguously prove it by means of a ROESY experiment. A strong cross-peak between H-3 and H-5 was observed, but not between H-3 and H-1.[9] The absolute stereochemistry of all amino acid units has been found to be *S*, and the Pro-thz unit exhibits *S* configuration as well. This was established after ozonolysis and/or acid hydrolysis followed by chiral HPLC analysis.[10]

We were not successful in assessing any bioactivity of lyngbyapeptin A (**3**). Our bioassays did not disclose antibacterial, antifungal, or protease-inhibiting activity.

The isolation of lyngbyabellin B (**2**) from a lyngbyabellin A-producing cyanobacterium is not surprising. Often, a series of compounds has been found in which one amino acid was substituted by another of similar hydrophobicity due to relaxed substrate specificity of some peptide synthetases.[11] The isolation of lyngbyapeptin A (**3**) from the same organism that already afforded a variety of interesting secondary metabolites[2,3,4] further demonstrates the biosynthetic potential of cyanobacteria.

## Experimental Section

**General Experimental Procedures.** $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ at 400/500 MHz and 100/125 MHz, respectively, using residual solvent signals as internal references. The HMQC experiments were optimized for $^1J_{CH} = 140$ Hz, and the HMBC experiments for $^nJ_{CH} = 7$ or 4 Hz. HRFABMS were recorded in the positive mode.

**Biological Material.** Cyanobacterium VP417 was identified as *L. majuscula* and collected several times since 1991 (before 1997 named VP496, VP503 or VP312) at Finger's Reef, Apra Harbor, Guam. The lyngbyabellin B-affording re-collection from January 19, 1999, was the largest up to now, from which we have already described the isolation of lyngbyabellin A (**1**).[3] A specimen of the cyanobacterium preserved in formalin has been deposited at the University of Hawaii.

**Extraction and Isolation.** Fractionation of the lipophilic extract of the recollection of VP417 from January 1999, has previously been reported.[3] Lyngbyabellin B (**2**) and lyngbyapeptin A (**3**) were isolated from a less polar fraction than lyngbyabellin A (**1**), eluting from Si gel with 5% *i*-PrOH in $CH_2Cl_2$ (51.2 mg). Semipreparative reversed-phase HPLC (Ultracarb, 5 ODS 30, 250 × 10 mm, 2 mL/min; UV detection at 220 nm) using an isocratic system of 80% aqueous MeCN gave impure **2** (1.4 mg, $t_R$ 15.4 min) and yielded **3** (15.7 mg, $t_R$ 24.5 min). Compound **2** was purified by reversed-phase HPLC on a different column (Hypersil, Phenyl-1, 10 μm, 250 × 10 mm, 2 mL/min; UV detection at 220 nm) under isocratic conditions (50% aqueous MeCN). Lyngbyabellin B (**2**) eluted at $t_R$ 12.3 min (0.7 mg).

**Lyngbyabellin B (2):** colorless amorphous solid, $[\alpha]^{25}_D$ −152° (*c* 0.06, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 200 (4.49), 224 (4.06), 236 (3.93) (sh) nm; IR (film) $\nu_{max}$ 3342, 2966, 2919, 2849, 1737, 1719, 1684, 1537, 1508, 1467, 1373, 1296, 1231, 1149,

967, 750, 691 $cm^{-1}$; $^1H$ NMR, $^{13}C$ NMR and HMBC data, see Table 1; FABMS *m/z* 679/681/683 (100:80:23, $[M + H]^+$ ion cluster), HRFABMS *m/z* $[M + H]^+$ 679.1778 (calcd for $C_{28}H_{41}Cl_2N_4O_7S_2$, 679.1794).

**Lyngbyapeptin A (3):** colorless oil, $[\alpha]^{25}_D$ −235° (*c* 0.58, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 201 (4.55), 225 (4.30), 240 (4.09) (sh) nm; IR (film) $\nu_{max}$ 2958, 2873, 1644 (br), 1513, 1456, 1247, 1177, 1106, 824, 735 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR, see Klein et al.[5]; HRFABMS *m/z* $[M + Na]^+$ 720.3788 (calcd for $C_{37}H_{55}N_5O_6S$ + Na, 720.3771).

**Acknowledgment.** Funding was provided by NCNPDDG grant CA53001 from the National Cancer Institute. The cyanobacterium was identified by Dr. G. M. L. Patterson, Department of Chemistry, University of Hawaii. Bioassays were performed by Dr. M. Lieberman, Department of Chemistry, University of Hawaii. Mass spectral analyses were conducted by the UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Riverside.

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(6) Conditions for the chiral HPLC analysis: column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$; flow rate, 0.8 mL/min; detection at 254 nm. The retention times ($t_R$, min) of the authentic amino acids were: Gly (7.8), L-valine (27.1), D-valine (49.2), L-cysteic acid (22.0), and D-cysteic acid (28.7). The retention times of the amino acid components in the acid hydrolyzate were 7.8, 22.0, 27.1, and 28.7 min, indicating the presence of glycine, L-valine, and both D- and L-cysteic acid. However, relative peak intensities at 22.0 and 28.7 min of 1:3 suggested that only partial racemization of the cysteine-derived unit had occurred under the applied conditions and that the original stereochemistry was D, corresponding to *S* configuration.
(7) This *Lyngbya* sp. was recently defined (Hoffmann, L.; Demoulin, V. *Belg. J. Bot.* **1991**, *124*, 82−88) to distinguish this reef-inhabiting cyanobacterium from other, more typical varieties of *L. majuscula*. The authors' description of *L. bouillonii* closely resembles the morphology of our collected cyanobacterium VP417, but we continue to use the widely accepted definition from Harvey ex Gomont, at least until genetic data are available and can be compared.
(8) Some conversion of enol ether **3** to the corresponding ketone could be observed.
(9) The numbering system from Klein et al.[5] has been adopted.
(10) Conditions for the chiral HPLC analysis,[6] barring the solvent, 2 mM $CuSO_4$−MeCN (95:5) for all amino acids, except for *N*,*O*-di-Me-Tyr, 2 mM $CuSO_4$−MeCN (90:10). The retention times ($t_R$, min) of the authentic amino acids were L-Pro (11.5), D-Pro (22.2), *N*-Me-L-Ile (26.7), *N*-Me-D-Ile (40.4), *N*-Me-L-*allo*-Ile (25.3), *N*-Me-D-*allo*-Ile (39.1), *N*-Me-L-Leu (46.0), *N*-Me-D-Leu (79.0), *N*,*O*-diMe-L-Tyr (81.5), and *N*,*O*-diMe-D-Tyr (87.5). The retention times of the amino acid components liberated in acid hydrolysis (6 N HCl at 110 °C, 12 h) were 26.7, 46.0, and 81.5 min, indicating the presence of *N*-Me-L-Ile, *N*-Me-L-Leu, and *N*,*O*-diMe-L-Tyr, respectively. A peak at 26.2 min (solvent mixture, 90:10), also observed when authentic *N*,*O*-diMe-L-Tyr was hydrolyzed under the same conditions, was attributed to *N*-Me-L-Tyr as a result of O-demethylation. When compound **3** was subjected to ozonolysis (−78 °C, $CH_2Cl_2$) prior to acid hydrolysis, an additional peak at 11.5 min could be detected in the HPLC trace caused by L-Pro liberated from the Pro-thz unit.
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NP000104N