Lyngbouilloside, a Novel Glycosidic Macrolide from the Marine Cyanobacterium *Lyngbya bouillonii*

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A glycosidic macrolide, lyngbouilloside (**1**), was characterized from the marine cyanobacterium *Lyngbya bouillonii* collected from Papua New Guinea. Its structure was elucidated by spectroscopic analysis and chemical derivatization. Relative stereochemistry was deduced from homonuclear and heteronuclear coupling constants as well as NOE information. Lyngbouilloside was modestly cytotoxic to neuroblastoma cells (IC_{50} value of 17 μM).

Marine cyanobacteria are an extraordinarily rich source of bioactive and structurally diverse secondary metabolites, most of which derive biosynthetically from a combination of the non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) pathways.[1] As part of our continued search for useful natural products from these life forms,[2] we encountered off the North Coast of Papua New Guinea an intriguing subtidal and filamentous species of *Lyngbya* having "cobweb-like" morphology. Laboratory examination identified it as the recently described species *Lyngbya bouillonii* Hoffmann and Demoulin (Oscillatoriaceae).[3] This species has quickly emerged as an exceptional source of new natural products, including a linear tetrapeptide (lyngbyapeptin)[4a] and four macrolides (lyngbyaloside (**2**), laingolide, laingolide A, and madangolide).[4b−d] A bioassay-guided fractionation of the organic extract of this recent collection resulted in the isolation of a new and bioactive macrolide glycoside, lyngbouilloside (**1**). This is the third report of a glycosidic macrolide isolated from a cyanobacterium;[4b,e] however, the close structural parallel between these cyanobacterial substances and several isolated from various marine invertebrates suggests that these latter compounds may also derive from cyanobacterial metabolism.

L. bouillonii was initially collected from Papua New Guinea in 1999 and its extract found to be cytotoxic as well as rich in secondary metabolites (NMR, TLC). A second, more extensive collection was made from the north coast of New Britain in 2000. The lipid extract (ca. 2.0 g) of this latter collection was fractionated using normal-phase silica gel vacuum-liquid chromatography through a stepwise gradient (hexanes/EtOAc/MeOH). Fractions eluting with 2% MeOH in EtOAc were found to be toxic to brine shrimp at 10 ppm, and these were subsequently pooled and fractionated using Mega Bond RP-18 Sep Pak. A cell toxic fraction (82% cytotoxicity at 10 μg/mL to the neuro-2a neuroblastoma cell line) was eluted with 10% H_2O in MeOH and then finally purified using reversed-phase HPLC [Phenomenex Sphereclone 5 $\mu\mu$ ODS (2) 250 × 10 mm, MeOH−H_2O (9:1)] to yield lyngbouilloside (**1**, 4.5 mg) (Figure 1).

Lyngbouilloside (**1**) gave a quasimolecular ion at *m/z* 607 ($[M + Na]^+$) by positive-ion FABMS and a $[M - H]^-$ peak at *m/z* 583 by negative-ion FABMS, thereby identifying **1** as possessing a molecular formula of $C_{31}H_{52}O_{10}$ (six degrees of unsaturation). This assignment was confirmed by HR-FABMS analysis. Four olefinic carbons and one carbonyl were observed in the ^{13}C NMR spectrum of **1**, accounting for three degrees of unsaturation; hence, lyngbouilloside was tricyclic. In addition, the IR spectrum of **1** indicated the presence of hydroxy (3461 cm^{-1}) and carbonyl (1696 cm^{-1}) groups. Upon acetylation of **1**, three diagnostic methyl singlets appeared at about 2 ppm in the ^1H NMR spectrum, indicating that **1** possesses three hydroxyl functionalities. By ^{13}C NMR analysis, the 10 oxygen atoms in **1** could be assigned to one ester, two methoxy ethers, one acetal, and one ketal, and eight additional carbons with only one attached oxygen atom (e.g., ether or hydroxy). The above oxygen atom count indicated that six were involved in forming connections between two different carbon atoms. A conjugated diene system was also present in lyngbouilloside as deduced from a UV absorbance at 235 nm.

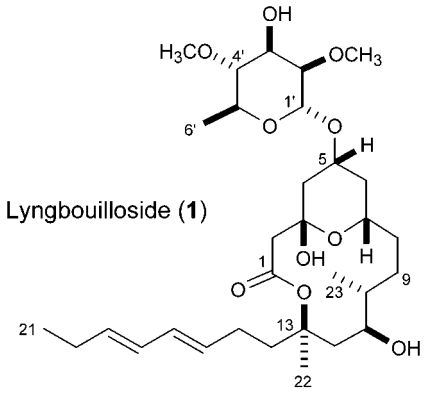

Figure 1. Structure of lyngbouilloside (**1**) [note that the relative stereochemistries of the aglycon and sugar portions have not been correlated, and depiction of the L-sugar is arbitrary].

The planar structure of lyngbouilloside (**1**) was established from analyses of one- and two-dimensional NMR data (Table 1). The presence of a 2,4-di-*O*-methylrhamnopyranoside moiety in **1** was revealed by vicinal $^3J_{H,H}$ coupling constants, correlations in the $^1H-^1H$ COSY and HMBC spectral data (Figure 2), and comparison with the same sugar unit in the sea-hare metabolite, auriside A (**4**, Figure 3).[5] Homonuclear coupling constant analysis and a ROESY correlation from H-1′ to *O*Me-2′ identified this sugar as the α-anomer (Figure 2).

For the aglycon moiety in **1**, four isolated proton spin systems were detected by $^1H-^1H$ COSY and, in combination with HMBC data, gave rise to substructures A−D (Figure 4 and Table 1). The *E,E* geometry of the conjugated diene system in partial structure B was deduced from

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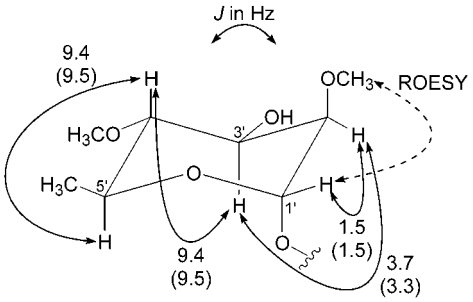

Figure 2. Coupling constant analysis of the 2,4-di-*O*-methylrhamnopyranoside moiety in lyngbouilloside (**1**) [coupling constants for the sugar unit in auriside A (**4**) are given in parentheses].[5]

proton−proton coupling constants of 14.6 and 14.6 Hz (in C_6D_6). Connection of units A through D was achieved by HMBC correlations between H-2ab/C-3, H-2ab/C-4, H-11/C-13, H-12a/C-13 and C-14, H-22/C-12 and C-14, H-14ab/C-12 and C-13, and H-22/C-1. The linkage between the aglycon portion and the 2,4-di-*O*-methylrhamnopyranoside unit was determined by HMBC correlations between H-5/C-1′ and H-1′/C-5, thereby establishing the planar structure of lyngbouilloside (**1**).

The conformation of the cyclic hemiketal moiety of **1** was determined by vicinal $^1H-^1H$ coupling constants. These dictated a chair conformation in the tetrahydropyran ring and that the substituents at C-5 and C-7 both be equatorial ($J_{4b,5} = J_{5,6b} = J_{6b,7} = 11.1$ Hz). The OH-3 group was positioned axially in order to accommodate a 2.3 Hz *W*-type coupling with H-4b. *J*-based configuration analysis was used to examine the relative stereochemistry at C-10 and C-11 and indicated an *erythro* relationship of substitutents at these two centers.[6] ROESY correlations were also observed between H_3-22 and H-12a, H-12a and H-11, and H-12a and H_3-23, indicating that these two methyl groups and H-11 lie on the same side of the macrocycle. Additional ROESY correlations between H-10 and H-7, and H-7 and H-5, allowed establishment of the relative stereochemisty of the aglycone portion of lyngbouilloside (**1**) as 3*S**,5*R**,7*S**,-10*R**,11*S**,13*R**. As indicated above, coupling constant analysis about the sugar ring allowed deduction of a 1′*R**,2′*R**,3′*R**,4′*S**,5′*S** relative stereochemistry. That only a single reliable NOE was observed between the aglycon and sugar portions of the molecule (H-5 and H-1′) precluded assignment of the relative configuration between these two sections [however, the L-sugar is depicted consistent with other members of this structure class (e.g., metabolites **2**, **4**−**6**].[4b,5,7,8] The limited supply of lyngbouilloside (**1**) prevented determination of its absolute stereochemistry.

Lyngbouilloside (**1**) represents only the second macrolide glycoside to be reported from a marine cyanobacterium [lyngbyaloside (**2**)[4b] was the first; acutiphycin (**3**) is from the freshwater cyanobacterium *Oscillatoria acutissima*].[4e] It is interesting to note that other glycosidic macrolides, such as the aurisides (e.g., **4**) from the sea-hare *Dolabella auricularia*[5] and the callipeltosides (e.g., **5**) from the sponge *Callipelta* sp.,[7] bear strong structural similarities to lyngbouilloside (**1**). These similarities suggest a possible cyanobacterial origin for these "marine invertebrate-derived" substances, either through sequestration in the diet (e.g., mollusks) or through symbiotic associations (e.g., sponges). In addition, structural similarities of **1** to the toxic

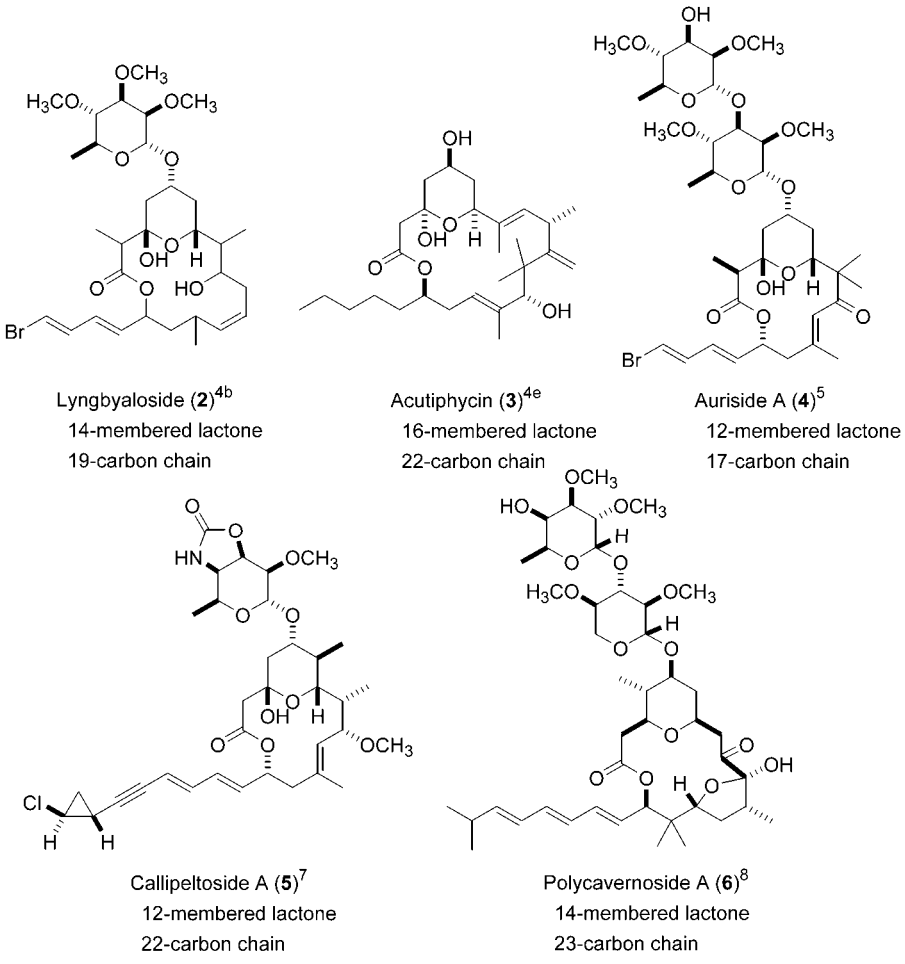

Figure 3. Marine glycosidic macrolides (**2**−**6**) related in structure to lyngbouilloside (**1**).

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Lyngbouilloside (**1**)[a]

position	^{1}H mult. (*J* in Hz)	^{13}C	HMBC[b]
1		172.9	
2a	2.52 d (12.2)	47.5	C-1, C-3, C-4
2b	2.39 d (12.2)		C-1, C-3, C-4, C-7
3		97.2	
OH-3	4.61 brd (2.3)		C-3, C-4
4a	2.13 m	41.9	C-3, C-5, C-6
4b	1.29 m		C-3, C-5, C-6
5	4.11 m	69.8	C-1′, C-4
6a	1.90 m	38.4	C-4, C-5
6b	1.18 dd (11.7)		C-4, C-5, C-7, C-8
7	3.79 m	70.2	
8a	1.71 m	31.9	C-7, C-9
8b	1.45 m		C-9
9a	1.49 m	33.0	C-10, C-11
9b	1.33 m		C-8, C-10
10	1.49 m	37.5	C-11, C-23
11	4.28 brd (5.9)	66.0	C-9, C-10, C-12, C-13, C-23
12a	2.78 d (15.5)	44.7	C-10, C-11, C-13, C-14, C-23
12b	1.46 m		
13		86.9	
14a	1.96 m	39.6	C-12, C-13, C-15, C-16, C-22
14b	1.63 m		C-12, C-13, C-15, C-16, C-22
15a	2.17 m	27.0	C-13, C-14, C-16
16	5.58 m	131.9	C-14, C-15, C-18
17	6.06 m	131.0	C-15, C-18, C-19
18	5.98 m	129.6	C-16, C-17, C20
19	5.61 m	134.8	C-17, C-20, C-21
20ab	2.07 m	26.0	C-18, C-19, C-21
21	0.99 t (7.4)	14.0	C-19, C-20
22	1.52 s	23.8	C-1, C-12, C-13, C-14
23	0.80 d (6.2)	14.0	C-9, C-10, C-11
1′	4.99 d (1.5)	94.2	C-5, C-2′, C-3′, C-5′
2′	3.38 dd (3.7, 1.5)	81.4	C-4′, C-3′, *O*Me-2′
3′	3.78 m	71.6	C-4′, C-5′
4′	2.94 t (9.4)	84.3	C-3′, C-5′, *O*Me-4′
5′	3.58 m	67.9	C-1′, C-3′, C-6′
6′	1.28 d (6.2)	18.2	C-4′, C-5′
*O*Me-2′	3.47 s	59.3	C-2′
*O*Me-4′	3.56 s	61.3	C-4′

[a] Spectra obtained at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in $CDCl_3$. [b] Data reported for experiments optimized at either 4 or 8 Hz.

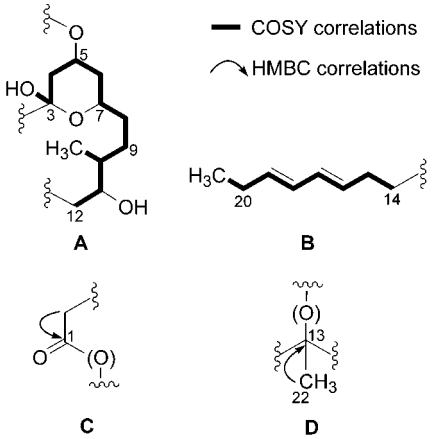

Figure 4. Partial structures **A**−**D** of lyngbouilloside (**1**).

polycavernosides (e.g., **6**), reported from the red alga *Polycavernosa tsudai*, provide further evidence to the possible cyanobacterial origin of these latter toxins as well.[8] However, lyngbouilloside (**1**) was only moderately cytotoxic to neuro-2a neuroblastoma cells (IC_{50} = 17 μM).[9]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV and IR spectra were recorded on Beckman DU 640B and Nicolet 510 spectrophotometers, respectively. NMR experiments were measured on a Bruker AM 400 MHz NMR spectrometer in $CDCl_3$ as an internal standard. Chemical shifts are reported in ppm, and coupling constants (*J*) are reported in Hz. Low- and high-resolution mass spectra were recorded on a Kratos MS50TC mass spectrometer. The isolation of lyngbouilloside (**1**) was performed on a Waters Millipore Model 590 pump and detected with a Waters Millipore Lambda-Max Model 480 LC spectrophotometer.

Biological Material. The first collection of *Lyngbya bouillonii* Hoffman and Demoulin was made from the Eastern Fields, Papua New Guinea (December 5, 1999) and given the collection number of PNGE9 5/Dec/99-1. A more extensive recollection of this cyanobacterium was made the following year from other locations in Papua New Guinea. In the second collection (August 21−27, 2000), samples of the cyanobacterium were hand collected at 8−15 m water depth using scuba from various reefs on the northern coast of New Britain, Papua New Guinea; Bangkok Pass (S 4°15.758′, E 151°28.547′), Father's Reef (S 4°55.153′, E 150°54.554′), May Reef (S 5°13.692′, E 150°30.078′), Unea Island (S 4°50.783′, E 149°09.174′), and Long Island (S 5°14.528′, E 147°02.058′). Upon collection, the pooled marine cyanobacterium was preserved in 50% ethanol−seawater and stored at low temperature until workup. Voucher specimens are available from W.H.G. as collection number PNGRD 21/Aug/00-2.

Extraction and Isolation. The thawed cyanobacterial material (PNGRD 21/Aug/00-2) was homogenized in CH_2Cl_2−MeOH (2:1, v/v) and filtered, and the solvents were removed in vacuo to yield a residue, which was partitioned between CH_2Cl_2 and H_2O. The marc was extracted repeatedly (× 4) with CH_2Cl_2−MeOH (2:1, v/v), and the combined CH_2Cl_2 layers were reduced in vacuo to yield 2 g of a dark green tar. The crude extract was fractionated using normal-phase silica gel (TLC grade) vacuum-liquid chromatography (VLC) through a stepwise gradient solvent system of increasing polarity starting from EtOAc in hexanes to EtOAc in MeOH. Fractions eluting with 2% MeOH in EtOAc were found to be active at 10 ppm in the brine shrimp toxicity assay. This fraction was refractionated using Mega Bond RP-18 Sep Pak and then HPLC using Phenomenex Sphereclone 5 μm ODS (2) (250 × 10.0 mm), MeOH−H_2O (82:18), to yield lyngbouilloside (**1**, 4.5 mg).

Lyngbouilloside (1): amorphous solid; $[\alpha]^{25}_D$ −38° (*c* 0.46, $CHCl_3$); UV (EtOH) λ_{max} 235 nm (ϵ 26 200); IR (neat) ν_{max} 3461, 2931, 1696, 1207, 1103, 1045, 755 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; LR-FABMS *m*/*z* 583 (19), 391 (12), 181 (39), 91 (100), 59 (38); HR-FABMS (negative ion, 3-nitrobenzyl alcohol) *m*/*z* 583.3486 (calcd for $C_{31}H_{51}O_{10}$ [(M − H)$^-$], 583.3482).

Acetylation of Lyngbouilloside (1). To 0.9 mg of **1** were added three drops of pyridine followed by six drops of acetic anhydride. The mixture was stirred in a vial at room temperature for 19 h, dried in vacuo, and prepared for ^{1}H NMR analysis: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, d, J = 6.2 Hz), 1.01 (3H, t, J = 7.4 Hz), 1.25 (1H, m), 1.32 (3H, d, J = 6.2 Hz), 1.30 (2H, m), 1.53 (3H, s), 1.54 (2H, m), 1.55 (2H, m), 1.72 (2H, m), 1.89 (2H, m), 2.01 (3H, s), 2.05 (2H, m), 2.06 (2H, m), 2.10 (1H, m), 2.15 (3H, s), 2.23 (3H, s), 2.40 (1H, d, J = 12.2 Hz), 2.62 (1H, d, J = 12.2 Hz), 2.90 (1H, d, J = 15.4 Hz), 3.22 (1H, t, J = 9.4 Hz), 3.47 (3H, s), 3.48 (3H, s), 3.49 (1H, m), 3.55 (1H, dd, J = 3.6, 1.5 Hz), 3.69 (1H, m), 3.75 (1H, m), 4.12 (1H, m), 4.62 (1H, d, J = 5.4 Hz), 4.95 (1H, d, J = 1.5 Hz), 5.10 (1H, dd, J = 8.4, 3.0 Hz), 5.55 (1H, m), 6.00 (2H, m).

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Supporting Information Available: FABMS and 1D and 2D NMR spectra of lyngbouilloside (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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