Lyngbyaloside, a Novel 2,3,4-Tri-*O*-methyl-6-deoxy-α-mannopyranoside Macrolide from *Lyngbya bouillonii* (Cyanobacteria)

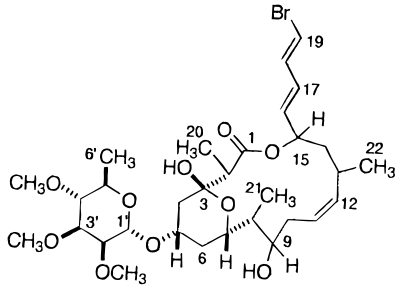
D. Klein,[†] J. C. Braekman,[†] D. Daloze,*[,†] L. Hoffmann,[‡] and V. Demoulin[‡]

Laboratory of Bio-organic Chemistry, Faculty of Sciences CP 160/07, University of Brussels, 50, Av. F. D. Roosevelt, B-1050 Brussels, Belgium, and Laboratory of Algology, Mycology and Experimental Systematics, Department of Botany, University of Liège, Sart Tilman B22, B-4000 Liège, Belgium

Received June 2, 1997[⊗]

A novel 16-membered macrolide linked to a 2,3,4-tri-*O*-methyl-6-deoxy-α-mannopyranoside, lyngbyaloside (**1**), has been isolated from the blue-green alga *Lyngbya bouillonii*, collected on Laing Island (Papua New Guinea). Its structure has been established by a detailed high-field 2D NMR study. The relative configuration of 8 out of the 13 stereogenic carbon atoms is proposed from the analysis of NOE difference experiments.

Cyanophyceae (Cyanobacteria or blue-green algae) constitute a rich source of novel bioactive metabolites with unprecedented structures.[1,2] In the course of a screening program to evaluate blue-green algae as a source of interesting bioactive metabolites, we reported the isolation of a novel macrolide, laingolide,[3] from the recently described *Lyngbya bouillonii* Hoffmann and Demoulin[4] (Oscillatoriaceae) collected at Laing Island in Papua New Guinea, the type locality. *L. bouillonii* is a filamentous, nonheterocystous, blue-green alga up to 50 μm wide which can easily be mistaken for *L. majuscula*, another large-sized blue-green alga often found in tropical regions and responsible for a dermatitis known as swimmer's itch. *L. bouillonii* is mainly found in coral reefs where it forms typical dark reddish, tenacious plant masses, strongly attached to madrepores in the infralittoral zone. The dried alga (11.9 g) was sequentially extracted with CH_2Cl_2 (0.04 g), EtOH (0.715 g), and MeOH (5.05 g). The two alcoholic extracts were pooled and partitioned with the mixture hexane:AcOEt:2-propanol:H_2O (3:4:2:6), an efficient solvent system for extracting lipidic material from a mixture. The less polar phase afforded 0.254 g, which was added to the 0.04 g of the CH_2Cl_2 extract, and this fraction was fractionated, first by chromatography over Sephadex LH-20 (EtOH), then over silica gel (CH_2Cl_2:MeOH) and, finally, by HPLC (reversed phase C-18, CH_3CN:H_2O), first from 6:4 to 10:0, then 65:35, isocratic. This procedure yielded 0.96 mg of a new compound which we have named lyngbyaloside (**1**).



1

The positive ion FABMS of **1** showed two quasi-molecular ions in a 1:1 ratio at *m/z* 685 and 683 ([M + Na]$^+$), accompanied by an [M + H − H_2O]$^+$ fragment at *m/z* 645 and 643, suggesting the presence of a bromine atom and a molecular weight of 662 (for ^{81}Br). In EIMS, the molecular ion was not detected. Instead, characteristic fragment ions were observed at *m/z* 644 and 642 [(M − H_2O)$^+$] ($C_{31}H_{47}O_9Br$ by HREIMS) and at *m/z* 457 and 455 [(M − $C_9H_{17}O_5$)$^+$] ($C_{22}H_{32}O_5Br$ by HREIMS). Thus, the molecular formula of **1** is $C_{31}H_{49}O_{10}Br$, implying 7 degrees of unsaturation.

The structure of **1**, including the relative configuration of 8 out of the 13 stereogenic carbon atoms, could be deduced by a complete 2D NMR study at 600 MHz (^{1}H/^{1}H COSY, TOCSY, HMQC, HMBC) and by NOE difference experiments. HMQC and HMBC experiments in $CDCl_3$ allowed us to detect 31 carbon atoms (Table 1), which included one carbonyl, six olefinic methines, one oxygenated quaternary carbon (δ 99.0), 12 methines [of which eight were oxygenated and one belonged to a ketal function (δ 95.0)], four methylenes, and seven methyls, three of which were methoxyl groups (δ_H 3.475, 3.48, and 3.54). One carbonyl and three double bonds accounted for the presence of 4 degrees of unsaturation, and thus, on the basis of its molecular formula, lyngbyaloside must be tricyclic. The UV spectrum in hexane showed absorptions at λ_{max} 199 (ε 10 700) and 240 (ε 25 700), indicating the presence of a conjugated diene probably bearing the bromine atom on the basis of the high absorbance values measured.[5] The connectivity from C-4 to C-19 was afforded by the ^{1}H/^{1}H COSY spectrum, which allowed us also to attach the methyl group at δ_H 0.91 to C-8 and that at δ_H 1.04 to C-13. The ^{1}H and ^{13}C chemical shifts indicated also the presence of one oxygen atom at C-5, C-7, C-9, and C-15. The configuration of the carbon−carbon double bond at C-11−C-12 was assigned as *Z* on the basis of the coupling constant $J_{11,12}$ (10.7 Hz). The exchangeable proton at δ 4.79 was coupled with a ^4J of 2.5 Hz to H4 at δ 1.13, and gave HMBC cross peaks with the carbons at δ 99.0 (C-3) and 39.5 (C-4). This allowed us to assign the quaternary carbon at δ 99.0 (C-3) to a hemiketal function and to connect it to C-4. These assignments were corroborated by a TOCSY experiment showing cross peaks between the δ 4.79 signal and those at 1.13 and 2.20 (H_2-4) and 4.12 (H-5). Moreover, partial structure **A** (Figure 1) could also be deduced from HMBC experiments. The quartet at δ_H 2.66 (J = 7.0

* To whom correspondence should be addressed. Phone: 32.2.6503537. Fax: 32.2.6502798. E-mail: ddaloze@ulb.ac.be.
[†] University of Brussels.
[‡] University of Liège.
[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1997.

S0163-3864(97)00275-9 CCC: $14.00

Table 1. NMR Data of Lyngbyaloside (**1**) ($CDCl_3$, 600 MHz, δ, J in Hz)

position	^{1}H	^{13}C[a]	HMBC[b]
1		177.5	
2	2.66, q, 7.0	47.6	C-1, C-3, C-20
3		99.0	
OH	4.79, d, 2.5		C-3[c], C-4[c]
4	2.20, dd, 12.0, 4.0	39.5	C-3, C-5, C-6
	1.13, t, 12.0		
5	4.12, m	69.0	C-3
6	2.20, dd, 12.0, 4.0	36.3	C-5
	1.02, q, 11.5		
7	3.73, bt, 11.0	70.5	
8	1.82, m	44.0	
9	3.87, bd, 10.6[d]	72.5	
10	2.13, m; 1.98, m	28.2	
11	5.40, dt, 10.7, 7.5	124.0	
12	5.37, t, 10.7	139.0	C-13
13	2.54, m	28.6	
14	1.65, ddd, 14.0, 10.0, 3.0	43.7	C-13
	1.42, bdd, 14.0, 11.0		
15	5.48, bdd, 11.0, 6.6	73.5	C-17
16	5.70, dd, 15.3, 6.5	132.0	C-15
17	6.13, dd, 15.3, 10.8	128.5	C-18, C-19
18	6.67, dd, 13.6, 10.8	136.3	
19	6.36, d, 13.6	110.2	C-17, C-18
20	1.18, d, 7.0	13.0	C-2, C-3
21	0.91, d, 7.0	11.5	C-7, C-9
22	1.04, d, 6.3	19.5	C-10, C-12, C-14
1′	5.01, d, 2.0	95.0	C-5, C-3′, C-5′
2′	3.52, dd, 2.7, 2.0[e]	77.5	
3′	3.44, dd, 9.3, 3.3	81.0	OMe-3′
4′	3.11, t, 9.3	82.0	C-3′, C-5′, C-6′, OMe-4′
5′	3.57, m	68.0	C-4′
6′	1.27, d, 7.0	17.5	C-4′, C-5′
OMe-2′	3.48, s	59.0	C-2′
OMe-3′	3.47, s	57.5	C-3′
OMe-4′	3.54, s	60.5	C-4′

[a] Obtained by HMQC and HMBC experiments. [b] Optimized for J = 5 Hz. [c] Optimized for J = 10 Hz. [d] In CD_3OD, dt, 11.0, 3.0. [e] Multiplicity determined in C_6D_6.

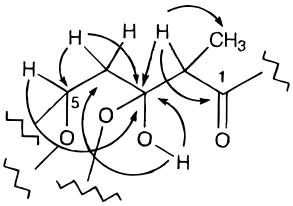


Figure 1. Partial structure **A** with key HMBC correlations.

Hz), which only coupled to the methyl group at δ_H 1.18 (δ_C 13.0), was deduced to be vicinal to a carbonyl group, as inferred from its chemical shift. Indeed, it gave HMBC cross peaks with the carbons at δ 177.5 (C-1), 99.0 (C-3), and 13.0 (20-Me), whereas the latter (δ_H 1.18) gave cross peaks with the carbons at δ 47.6 (C-2) and 99.0 (C-3). On the other hand, H-4$_{eq}$ (δ 2.20) and H-5 (δ 4.12), both gave cross peaks with the C-3 hemiketal carbon. These results allowed us to assign the δ 2.66 signal to H-2 and to connect the C-1−C-2 moiety to C-4 through the C-3 hemiketal carbon.

The chemical shift of H-15 (δ 5.48) strongly suggested that it is part of an ester linkage, and thus the C-15 oxygen was linked to the C-1 carbonyl to afford a 16-membered macrolide. No HMBC correlation was observed between H-15 and C-1, but this is also the case in the related glycoside macrolides callipeltosides A,[6] B, and C.[7] The location of the hemiketal ring between C-3 and C-7 was deduced from NOE difference spectra. Irradiation of H-7 at δ 3.73 brought about enhancements of the OH signal at δ 4.79 and the H-5 signal at

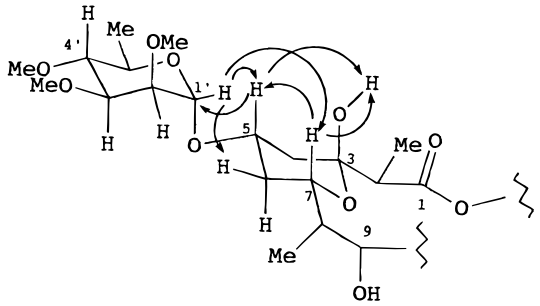


Figure 2. Key NOE enhancements observed in NOE difference experiments on **1**.

δ 4.12. Conversely, irradiation of H-5 (δ 4.12) led to enhancements of the OH signal at δ 4.79 and the H-7 signal at 3.73 (Figure 2). These data not only fixed the hemiketal ring position, but suggested also the presence of a six-membered ring with OH-3, H-5, and H-7 in axial positions. Large vicinal coupling constants for H-5 and H-7 with their axial neighbors fully confirmed this assignment ($J_{4ax,5}$ = 12.0, $J_{5,6ax}$ = 11.5, and $J_{6ax,7}$ = 11.0 Hz). The low-field chemical shift of OH-3 (δ 4.79), together with the fact that it appears as a doublet due to a coupling with H_{4ax}, strongly suggested that it is engaged in an intramolecular hydrogen bond, presumably with the carbonyl group at C-1. The structure of the macrolide portion of the molecule was completed by attaching a four-carbon side chain to C-15, corresponding to a (*E*,*E*)-1-bromobuta-1,3-diene moiety (^{1}H/^{1}H COSY correlation H-15/H-16 and TOCSY correlations H-15/H-16/H-17/H-18/H-19). This assignment was confirmed by the UV spectrum (see the Experimental Section) and by the chemical shifts and coupling constants of H-16 to H-19, in comparison with literature models.[5] Particularly informative were the $J_{16,17}$ and $J_{18,19}$ values (15.3 and 13.8 Hz, respectively), and the chemical shift of the bromine-bearing carbon (C-19, δ 110.2).

The analysis of the NMR spectrum of the glycosidic portion was pursued starting from the proton doublet at δ 5.01 (J = 2.0 Hz), attached to a ketal carbon (95.0, C-1′). The ^{1}H/^{1}H COSY and TOCSY spectra established the connectivities from H-1′ to H_3-6′. The three methoxyl groups at δ 3.48, 3.47, and 3.54 were attached to C-2′, C-3′, and C-4′, respectively, from their cross peaks in HMBC with the carbon signals at 77.0, 81.0, and 82.0. These data, together with the coupling constants of H-2′/H-3′/H-4′/H-5′ (see Table 1), led us to formulate the remaining portion of the molecule as 2,3,4-tri-*O*-methyl-6-deoxy-α-mannopyranoside. As expected, the base peak in the positive-ion FABMS appeared at m/z 189 ($C_9H_{17}O_4$ by HREIMS).

The structure of lyngbyaloside was completed by connecting the 2,3,4-tri-*O*-methyl-6-deoxy-α-mannopyranose moiety to C-5 of the macrolide through a glycoside linkage, by observation of HMBC correlations between H-1′ and C-5 and between H-5 and C-1′. Moreover, strong NOE effects between the anomeric proton at δ 5.01, H-5 (δ 4.12), H-7 (δ 3.73), and H-6$_{eq}$ (δ 2.20) suggested that the sugar and macrolide portions have the spatial relationship depicted in Figure 2. Taking into account the molecular formula of **1**, the attachment of the sugar moiety to C-5 of the macrolide implied that the oxygen function at C-9 was an hydroxyl group. In this context, we noted that H-9 appeared as

a broad doublet in the ^{1}H NMR spectrum measured in $CDCl_3$ (J = 10.6), but as a well-resolved dt (J = 11.0, 3.0) in CD_3OD, presumably because of the H/D exchange.

Due to the flexibility of the 16-membered macrolide ring, we could not unambiguously assign the relative configurations of the five stereogenic carbon atoms belonging to that ring. This problem will be addressed in the near future.

To the best of our knowledge, this is the first isolation of a glycoside macrolide from a blue-green alga. Several structural features of lyngbyaloside **1**, such as the presence of a 16-membered macrolide linked to an unusual sugar, a hemiketal function between C-3 and C-7 of the macrolide ring, and a diene side chain bearing a halogen atom, are common with callipeltosides A, B, and C, cytotoxic 14-membered glycoside macrolides, recently reported from a lithistid sponge by Zampella *et al.*[6,7] These authors suggested that these glycoside macrolides are probably of microbial origin. Our isolation of lyngbyaloside (**1**) from a blue-green alga brings strong support to this view. Due to the small amounts of material available, no bioassays have yet been performed on lyngbyaloside. In view of the new and interesting structure of this compound, we plan to recollect a larger sample of *L. bouillonii*, so as to be able to completely determine its relative configuration and to evaluate its biological properties.

Experimental Section

General Experimental Procedures. UV spectra were taken on a Philips PU 8700 UV−vis spectrophotometer in hexane. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. EIMS, HREIMS, and positive-ion FAMS measurements were performed on a Fisons VG Autospec. The NMR spectra were recorded in $CDCl_3$, C_6D_6, or CD_3OD at 600 MHz (Varian Unity 600 instrument). The chemical shifts (δ) are reported in ppm and the coupling constants in hertz. Flash liquid chromatography was performed over Macherey-Nagel Si gel (0.04−0.063 mm) and thin layer chromatography analyses (TLC) on Polygram SilG/UV_{254} precoated plates (0.25 mm). HPLC separations were performed on a Waters LCM1 plus apparatus coupled to a Waters 996 photodiode array detector, using a Merck Lichrocart 250-10 Lichrospher (10 μm) column (flow, 5 mL/min).

Biological Material. The algal material was collected by SCUBA diving in August and October 1995, as well as in December 1996 at a depth of 1−10 m on the coral reef of Laing Island (Madang Province) located on the northern coast of Papua New Guinea. The material was air-dried or dried with moderate heat and stored in plastic bags with silica gel until the return to the laboratory. A voucher specimen is maintained in the collections of the Laboratory of Algology, Mycology and Experimental Systematics at the University of Liège.

Isolation of Lyngbyaloside (1). The dried material (11.9 g) was sequentially extracted three times with CH_2Cl_2, EtOH, and five times with MeOH, affording 0.04, 0.715, and 5.05 g, respectively, after evaporation of the solvent. The two alcoholic extracts were pooled and partitioned with hexane:AcOEt:2-propanol:H_2O (3:4:2:6), and the material present in the organic phase (0.254 g) was joined to the 0.04 g of the CH_2Cl_2 extract. This fraction was chromatographed over Sephadex LH-20 with EtOH to afford seven fractions. Fraction D (83.2 mg) was submitted to a silica gel column chromatography (CH_2Cl_2:MeOH, from 100/0 to 0:100), and the fractions eluted with CH_2Cl_2:MeOH (96:4) were pooled and treated with ethereal CH_2N_2. The fatty acid methyl esters thus obtained were removed by passage over a Sep-pack column (hexane:AcOEt (9:1) then CH_2Cl_2:MeOH (9:1)), and final purification was made by reversed phase HPLC with CH_3CN:H_2O, first from 60:40 to 100:0 in 15 min, then with CH_3CN:H_2O (65:35), isocratic. This procedure afforded 0.96 mg of lyngbyaloside (**1**) as an amorphous solid. UV (hexane): λ_{max} 199 (ϵ 10 700) and 240 nm (ϵ 25 700). NMR data: see Table 1. EIMS (70 eV): m/z $[M]^+$ not detected; $[M - H_2O]^+$ 644 (0.05), 642 (0.05), 581 (0.075), 563 (0.062), 550 (0.05), 530 (0.14), 528 (0.145), 512 (0.075), 485 (1.3), 483 (1.3), 457 (3.75), 455 (3.75), 315 (7.5), 313 (7.5), 189 (70), 101 (60), 88 (100). HREIMS: m/z 642.2380 (calcd for $C_{31}H_{47}O_9{}^{79}Br$, 642.2403); 485.1363 (calcd for $C_{23}H_{32}O_6{}^{81}Br$, 485.1362); 483.1387 (calcd for $C_{23}H_{32}O_6{}^{79}Br$, 483.1382); 457.1407 (calcd for $C_{22}H_{32}O_5{}^{81}Br$, 457.1412); 455.1424 (calcd for $C_{22}H_{32}O_5{}^{79}Br$, 455.1433); 189.1120 (calcd for $C_9H_{17}O_4$, 189.1127). Positive-ion FABMS: m/z $[M + Na]^+$ 685 (0.36), 683 (0.34), $[M + H - H_2O]^+$ 645 (1.12), 643 (1.12), 439 (5.8), 437 (5.8), 277 (5.5), 268 (4.8), 207 (4.4), 189 (100).

Acknowledgment. We thank the "Fonds de la Recherche Fondamentale Collective" (FRFC) for financial support (contracts 2.4570.93-96 and 2.4502.95). We also thank Mr. C. Maerschalk for the NMR spectra and Mr. C. Moulard for the mass spectra and G. Castillo for collecting part of the material. L.H. is Research Associate of the Belgian Fund for Scientific Research. This paper represents contribution no. 344 of King Leopold III Biological Station, Laing Island, Papua, New Guinea.

References and Notes

(1) Moore, R. E.; Ohtani, I.; Moore, B. S.; Dekoning, C. B.; Yoshida, W. Y.; Runnegar, M. T. C.; Carmichael, W. W. *Gazz. Chim. Ital.* **1993**, *123*, 329−336.
(2) Patterson, G. M. L.; Larsen, L. K.; Moore, R. E. *J. Appl. Phycol.* **1994**, *6*, 151−157.
(3) Klein, D.; Braekman J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *Tetrahedron Lett.* **1996**, *37*, 7519−7520.
(4) Hoffmann, L.; Demoulin, V. *Belg. J. Bot.* **1991**, *124*, 82−88.
(5) Fusetani, N.; Li, H.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49*, 1203−1210.
(6) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085−11088.
(7) Zampella, A.; D'Auria, M. V.; Minale, L. *Tetrahedron* **1997**, *53*, 3243−3248.

NP9702751