Madangolide and Laingolide A, Two Novel Macrolides from *Lyngbya bouillonii* (Cyanobacteria)

D. Klein,† J. C. Braekman,*,† D. Daloze,*,† L. Hoffmann,‡ G. Castillo,‡ 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 January 25, 1999

Two new macrolide derivatives, madangolide (**2**) and laingolide A (**3**), have been isolated from the cyanobacterium *Lyngbya bouillonii*, collected in Papua New Guinea. Their structures (without stereochemistry) have been established by detailed high-field 1D and 2D NMR studies and, in the case of **3**, by comparison with the spectroscopic data of laingolide (**1**), previously isolated from the same organism.

Cyanophyceae (Cyanobacteria) 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 two novel macrolides, laingolide (**1**)[3] and lyngbyaloside,[4] as well as a modified linear tetrapeptide, lyngbyapeptin A,[5] from the recently described *Lyngbya bouillonii* Hoffmann and Demoulin[6] (Oscillatoriaceae). This organism proves to be an exceptionally rich source of diverse secondary metabolites, and we now report the isolation and structure determination of two further macrolides, madangolide (**2**) and laingolide A (**3**), from another collection made in the vicinity of Madang (north coast of Papua New Guinea) (Figure 1).

Madangolide (**2**) (1.7 mg) and laingolide A (**3**) (1.5 mg) were isolated from the dried alga (21.7 g) by the procedure outlined in the Experimental Section. The HREIMS of madangolide (**2**) showed a molecular ion at *m*/*z* 377.2926, corresponding to the molecular formula $C_{23}H_{39}NO_3$ (calcd as 377.2930, $\Delta = 0.4$ mDa). The IR spectrum showed the presence of ester (ν_{CO} 1729 cm^{-1}) and amide (ν_{CO} 1662 cm^{-1}) functionalities, whereas the UV spectrum indicated the presence of a conjugated system [λ_{max} (MeOH): 200 (ϵ 16 600) and 244 nm (ϵ 24 400)]. The structure of **2** could be deduced by 1D and 2D NMR studies at 600 MHz (^{1}H, ^{13}C, ^{1}H/^{1}H COSY, HMQC, HMBC). The ^{13}C NMR spectrum of **2** displayed 23 carbon atom signals (Table 1), which included two carbonyls, four olefinic methines, one quaternary carbon (δ 36.0), four sp^3 methines, one bearing an oxygen atom (δ 78.0), five methylenes, and seven methyls, three of which belonged to a *tert*-butyl group (δ 26.7) and one to a *N*-methyl function (δ 31.0). Two carbonyl groups and two carbon−carbon double bonds accounted for the presence of four degrees of unsaturation, and thus, on the basis of its molecular formula, **2** must be monocyclic. The connectivity from C-2 to C-7 and from C-10 to C-16 was afforded by the ^{1}H/^{1}H COSY spectrum, which allowed us also to attach the methyl groups at δ 1.05, 0.91, and 1.16 to C-2, C-5, and C-10, respectively. The location of the *tert*-butyl group at C-7 followed from HMBC correlations between H-7, C-20 and C-21, whereas HMBC correlations between the carbonyl carbon at δ 175.6, H-7 and H$_3$-22 fixed the position of the lactone group (Table 1). The

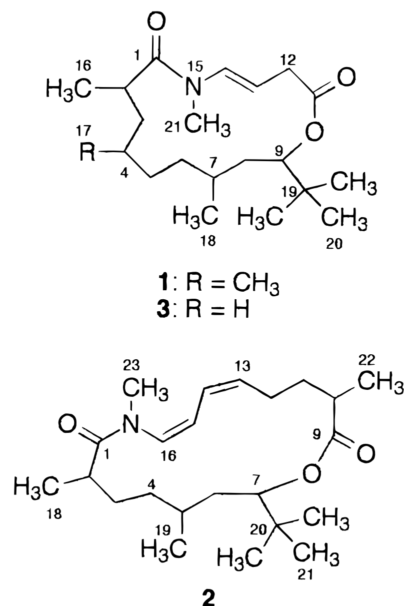

Figure 1. Structures of laingolide (**1**), madangolide (**2**), and laingolide A (**3**).

presence of a lactam, which is part of a dienamide system, was deduced from diagnostic HMBC correlations between the *N*-CH$_3$ group, the C-1 carbonyl group at δ 177.4 and the C-16 vinyl carbon at δ 130.2. These correlations allowed us to attach the nitrogen atom to C-16, and to close the ring between the *N*-CH$_3$ moiety and the carbonyl group at δ 177.4. The configuration of the Δ^{15} double bond was easily assigned as *Z* on the basis of the coupling constant ($J_{15,16}$ = 6.6 Hz). This assignment was more difficult for the Δ^{13} double bond. The H-14 and H-15 signals were indeed nearly superimposed, whereas the strong couplings between H-13 and both H-12 precluded the direct determination of $J_{13,14}$. This problem was solved by selective irradiation of the two H-12 signals at δ 2.23 and 2.32, upon which the H-13 multiplet collapsed to a doublet, $J_{13,14} = 9.0$ Hz. This result allowed us to assign the *Z* configuration to the Δ^{13} double bond as well. Owing to the conformational mobility of the seventeen-membered ring of **2**, and the small amount of material available, the relative configuration of the four stereogenic carbon atoms could not be assigned.

Laingolide A[7] (**3**) possessed the molecular formula $C_{20}H_{35}NO_3$, as shown by HREIMS [M$^+$ at *m*/*z* 337.2614; calcd 337.2617 ($\Delta = 0.3$ mDa)] and thus differed from laingolide

* To whom correspondence should be addressed. Tel.: +32 2 650 3537 and 650 2961. Fax: +32 2 650 2798. E-mail: ddaloze@ulb.ac.be and braekman@ulb.ac.be.
† University of Brussels.
‡ University of Liège.

10.1021/np9900324 CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/04/1999

Table 1. NMR Data of Madangolide (**2**) ($CDCl_3$, 600 and 150.87 MHz, δ, J in Hz)

position	^1H	^{13}C	HMBC[a]
1		177.4	
2	2.67, sex, 7.0	37.1	C1, C-3, C-18
3a	1.64, m	32.2	C-1, C-2
3b	1.23, m		C-1, C-18
4a	1.23, m	33.8	
4b	1.00, m		C-3
5	1.50, m	31.0	C-19
6a	1.50, m	36.8	
6b	1.48, m		C-4, C-5, C-7, C-19
7	4.81, dd, 7.8, 3.0	78.0	C-5, C-9, C-20, C-21
9		175.6	
10	2.52, dquin, 7.0, 2.5	39.5	C-22
11a	1.88, m	32.6	C-9, C-22
11b	1.62		C-9, C-10, C-12, C-13
12a	2.32, m	25.9	C-11, C-13, C-14
12b	2.23, m		C-10, C-11, C-13
13	5.64, m	135.9	
14	5.97, m	123.6	C-12, C-13, C-16[b]
15	5.99, m	121.3	C-12, C-13, C-16[b]
16	6.14, bd, 6.6	130.2	C-14, C-15
18	1.05, 3H, d, 6.6	17.1	C-1, C-2, C-3
19	0.91, 3H, d, 6.0	20.3	C-4, C-5, C-6
20		36.0	
21	0.86, 9H, s	26.7	C-6, C-7, C-20
22	1.16, 3H, d, 7.0	16.9	C-9, C-10, C-11
23	3.01, 3H, s	35.4	C-1, C-16

[a] Optimized for $J = 5$ and 10 Hz. [b] Correlations of H-14 and H-15 could not be distinguished.

Table 2. NMR Data of Laingolide A (**3**) and ^{13}C NMR of Laingolide (**1**)[3] ($CDCl_3$, 600 and 150.87 MHz, δ, J in Hz)

	3			1
position	^1H	^{13}C	HMBC[a]	^{13}C
1		176.4		176.3
2	2.99, m	36.2		34.5
3a	1.56, m	36.6	C-1, C-2, C-4, C-16	40.8
3b	1.40, m		C-1, C-2, C-4	
4a	1.24, m	26.2	C-3	31.3
4b	1.04, m		C-16	
5a	1.26, m	26.8	C-6	33.7
5b	1.12, m			
6a	1.32, m	36.8	C-5	33.3
6b	1.23, m		C-7	
7	1.17, m	27.7		28.5
8a	1.58, m	35.5		35.4
8b	0.99, m		C-17	
9	4.81, dd, 11.0, <1.0	79.7	C-8, C-11, C-19	79.1
11		172.5		171.7
12a	3.06, ddd, 12.0, 6.0, 1.5	37.6	C-11	36.3
12b	2.94, dd, 12.0, 10.8		C-11, C-13, C-14	
13	5.18, ddd, 14.0, 10.8, 6.0	105.0	C-12	104.8
14	7.01, dd, 14.0, 1.5	133.6	C-1, C-11, C-12, C-20	131.9
16	1.16, 3H, d, 6.6	18.5	C-1, C-2	18.5
17	0.83, 3H, d, 7.0	21.2	C-6, C-7, C-8	20.6[b]
18		35.1		20.6
19	0.89, 9H, s	26.7	C-9, C-18, C-19	34.3
20	3.10, 3H, s	31.2	C-1, C-13, C-14	25.9
21				30.6

[a] Optimized for $J = 5$ Hz. [b] From this position, the numbering of **1** and **3** differs by one C atom.

(**1**) only by having one carbon and two hydrogen atoms less. The assignment of all hydrogen and carbon signals of the molecule was performed by 1D and 2D NMR experiments (^1H, $^1H/^1H$ COSY, HMQC, HMBC). We should mention that the sample underwent a partial degradation during the NMR measurements, so that the interpretation of the data was complicated by the presence of signals due to impurities. However, the comparison of the ^{13}C NMR data with those of laingolide (**1**)[3] (Table 2) clearly showed that **3** possessed the same fifteen-membered macrocyclic ring as **1**, as well as the lactone, enamide, and *tert*-butyl functionalities, but lacked one of the methyl groups of **1**. The close similarity between the carbon atom signals of C-7 to C-14 in both compounds (Table 2) strongly suggested that the missing carbon atom was the one fixed at C-2 or C-4 of **1**. Arguments in favor of the latter hypothesis came from the assignment of the H_2C-2 to H_2C-6 signals. This was initially not straightforward, owing to the superimposition of several hydrogen signals (e.g. H-4a, H-5a, H-6b). All these data pointed to structure **3** for laingolide A. This hypothesis was strengthened by calculating the chemical shifts of C-2 to C-7 of **3**, starting from the values measured for the corresponding carbon atoms of **1**, and using the substituent parameters computed for the introduction of a CH_3 group as a branch in an alkane (α, +6; β, +8; γ, −2).[8] This approach gave the following results (calculated values in brackets): C-2, 36.2 (36.4); C-3, 36.6 (32.8); C-4, 26.2 (25.2); C-5, 26.8 (25.5); C-6, 36.8 (35.1); C-7, 27.7 (28.3). Of course, the substituent parameters used do not take into account the proximity effects that could arise in a system such as **1** (e.g. between CH_3-16 and CH_3-17). Nevertheless, the comparison between calculated and measured δ showed that they are in agreement, except for C-3, which is not unexpected as it is the sole carbon atom located between two $CH-CH_3$ groups. As for **2**, the relative configuration of **3** could not be determined.

In view of the new and interesting structure of these compounds, we plan to recollect a larger sample of *Lyngbya bouillonii*, so as to be able to determine their relative configurations and to evaluate their biological properties.

Experimental Section

General Experimental Procedures. UV spectra were taken on a Philips PU 8700 UV−vis spectrophotometer in methanol. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. EIMS and HREIMS measurements were performed on a Fisons VG Autospec. The NMR spectra were recorded in $CDCl_3$ at 600 MHz (Varian Unity 600 instrument). The chemical shifts (δ) are reported in ppm and the coupling constants in Hz. 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) or a 250-10 Chrompack Chromspher 5 C-18 column (flow: 5 mL/min).

Biological Material. The algal material was collected by SCUBA diving at a depth of 1−10 m on the coral reefs near Madang (July 1998) on the northern coast of Papua New Guinea. The material was air-dried or dried with moderate heat and stored in plastic bags with Si 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 (LG).

Isolation of Madangolide (2) and Laingolide A (3). The dried material (21.7 g) was sequentially extracted three times with CH_2Cl_2, EtOH, and five times with MeOH, affording 0.131, 3.8, and 5.2 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). The material present in the organic phase (0.536 g) was added to the 0.131 g of the CH_2Cl_2 extract. This fraction was chromatographed over Sephadex LH-20 with EtOH, to afford 10 fractions. Fraction D (0.160 g) was submitted to a Si gel column chromatography (CH_2Cl_2−MeOH, from 100:0 to 0:100), and the fraction eluting with CH_2Cl_2−MeOH 95:5 was submitted to RP-C_{18} chromatography with CH_3CN as eluent. This afforded 0.064 g of a mixture that was treated with ethereal CH_2N_2 and chromatographed on a Si gel column, using

successively hexane−AcOEt 9:1 and CH_2Cl_2−MeOH 9:1 as eluent. The CH_2Cl_2−MeOH fractions were pooled, affording 0.028 g of material that was submitted to final reversed-phase separation on a Chromspher 5 C_{18} column (CH_3CN−H_2O, from 50:50 to 100:0), to afford madangolide (**2**) (1.7 mg) and laingolide A (**3**) (1.5 mg), as amorphous solids.

2: UV and IR, see text; NMR, see Table 1; HREIMS *m*/*z* 377.2926 (calcd for $C_{23}H_{39}NO_3$, 377.2930); 304.2640 (calcd for $C_{20}H_{34}NO$, 304.2640); 182.1179 (calcd for $C_{10}H_{16}NO_2$, 182.1181); EIMS (70 eV) *m*/*z* 377 (67, M^+); 320 (14), 313 (18), 304 (45), 250 (10), 182 (100), 166 (88), 110 (15), 109 (15), 96 (80), 94 (35), 83 (37).

3: NMR, see Table 2; HREIMS *m*/*z* 337.2614 (calcd for $C_{20}H_{35}NO_3$, 337.2617); 252.2329 (calcd for $C_{16}H_{30}NO$, 252.2327); 115.0634 (calcd for $C_5H_9NO_2$, 115.0633); EIMS (70 eV) *m*/*z* 337 (27, M^+); 252 (8); 140 (6); 127 (7); 115 (41); 97 (23); 83 (14); 75 (12); 70 (100); 55 (31).

Acknowledgment. We thank the Fonds de la Recherche Fondamentale Collective (FRFC) for financial support (Contracts No. 2.4570.93-96, 2.4502.95, 2.9001.90, and 2.4521.96). We also thank Mr. C. Maerschalk and Dr. M. Luhmer for the NMR spectra and Mr. C. Moulard for the mass spectra. L.H. is Research Associate of the Belgian National Fund for Scientific Research. One of us (D.K.) thanks the Fonds van Buuren for financial support.

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) Klein, D.; Braekman, J. C.; Daloze, D.; Hoffmann, L.; Demoulin, V. *J. Nat. Prod.* **1997**, *60*, 1057−1059.
(5) Klein, D.; Braekman J. C.; Daloze, D.; Castillo, G.; Hoffmann, L.; Demoulin, V. *Tetrahedron Lett.* **1999**, *40*, 695.
(6) Hoffmann, L.; Demoulin, V.*Belg. J. Bot.* **1991**, *124*, 82−88.
(7) We do not use the name "4-demethyllaingolide" for compound **3**, as we have no proof as yet that laingolide (**1**) and **3** have the same relative configuration.
(8) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: London, 1978; pp 36−37.

NP9900324