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TWO QUINOLINE ALKALOIDS FROM THE CARIBBEAN CYANOBACTERIUM *LYNGBYA MAJUSCULA*

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Key Word Index—*Lyngbya majuscula*; Oscillatoriaceae; cyanobacteria; blue-green alga; quinoline alkaloid; 4,8-dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline; 4,8-dimethyl-6-hydroxyquinoline.

Abstract—Fractionation of the lipid extract of the marine cyanobacterium *Lyngbya majuscula* collected from Curaçao afforded two quinoline alkaloids in low yield. Their structures were determined as 4,8-dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline and 4,8-dimethyl-6-hydroxyquinoline on the basis of spectroscopic analysis, mainly 2D NMR spectroscopy. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In our continuing search for bioactive secondary metabolites from marine cyanobacteria, we have undertaken an in-depth investigation of a Caribbean variety of *Lyngbya majuscula* (family Oscillatoriaceae). The lipid extract of this Curaçao collection of *L. majuscula* was found to possess several different bioactivities. Bioassay guided fractionation led to the previous isolation of four bioactive natural products (curacin A, malyngamide H, antillatoxin, and barbamide [1–4]). In the course of the isolation of these bioactive components, two new alkaloids were obtained in small yield from non-bioactive fractions. This paper focuses on the isolation and structure determination of these two related 6-hydroxyquinoline alkaloids (**1**, **2**).

RESULTS AND DISCUSSION

The lipophilic extract (2:1 CH_2Cl_2–MeOH) was fractionated by a combination of chromatographic methods on silica gel, Sephadex LH-20 and ODS silica gel to afford two new quinoline alkaloids **1** and **2**. Compound **1** was obtained in low yield as a white amorphous powder (1.5 mg, 0.05% of extract). The HR-EI-mass spectrum of **1** gave a $[M]^+$ peak at *m/z* 333.1578 consistent with the molecular formula $C_{18}H_{23}NO_5$. The IR spectrum showed the presence of hydroxyl (3602 cm^{-1}) and aromatic (1620, 1407 cm^{-1}) moieties, and the UV spectrum exhibited four absorption maxima at 236, 286, 316, and 328 nm (log ε 4.65, 3.57, 3.44, and 3.44).

The ^1H NMR spectrum of compound **1** in $CDCl_3$ was complicated by extensive overlap (see Table 1), and could be simplified using C_6D_6 as solvent. In this latter solvent, all of the protons were well dispersed and the connectivities, as well as the coupling constants, were easily determined. Of the eight degrees of unsaturation implied by the molecular formula, seven could be accounted for by examination of the ^{13}C NMR spectral data as a 4-, 6-, 8-trisubstituted quinoline moiety (Table 1); hence **1** had an additional ring system.

From the COSY and ^1H-^{13}C-COSY of compound **1**, it was possible to establish the nature of the substituents at the 4- and 8-positions, as well as the remaining ring system. The substituent at C-4 was delineated by long range homonuclear correlation cross peaks between the methyl protons at δ 2.17 and H-3 (δ 6.70), which in turn showed a cross peak to H-2 (δ 8.68). In a similar manner, the substituent at C-8 was defined by the long range correlation between the methyl protons at δ 2.94 and H-7 (δ 7.41), which in turn showed a *meta* coupling to H-5 (δ 7.38). Further, a third spin system was terminated by a resonance characteristic of an anomeric proton (H-1′, δ 5.02; C-1′, δ 102.3) and possessed sequential couplings to H-2′ (δ 3.36), H-3′ (δ 3.69), H-4′ (δ 3.20), and H_a-5′ (δ 3.83) and H_b-5′ (δ 3.11). These data described the coupling pattern for a xylopyranose moiety as the remaining substituent at the 6-position of the quinoline ring and accounted for all of the atoms in com-

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Table 1. ^1H and ^{13}C NMR chemical shift data (400 MHz) for compounds **1** and **2**

Position	**1** ($CDCl_3$) δ_H	**1** ($CDCl_3$) δ_C	**1** (C_6D_6) δ_H	**1** (C_6D_6) δ_C	**2** (MeOD-d_4) δ_H	**2** (MeOD-d_4) δ_C
2	8.70 *d* (4.3)	147.4 *d*	8.68 *d* (4.2)	147.5 *d*	8.67 *d* 4.4	145.3 *d*
3	7.21 *d* (4.3)	122.2 *d*	6.70 *d* (4.3)	122.3 *d*	7.16 obs.	121.5 *d*
4	—	143.4 *s*	—	142.7 *s*	—	141.8 *s*
4a	—	129.1 *s*	—	129.4 *s*	—	129.9 *s*
5	7.33 obs.*	105.5 *d*	7.38 *br s*	106.0 *d*	7.11 *d* 1.5	102.8 *d*
6	—	154.1 *s*	—	154.9 *s*	—	155.1 *s*
7	7.33 obs.	121.7 *d*	7.41 *d* (2.1)	121.1 *d*	7.16 obs.	121.7 *d*
8	—	139.9 *s*	—	140.7 *s*	—	138.3 *s*
8a	—	144.1 *s*	—	144.9 *s*	—	143.6 *s*
9	2.64 *br s*	19.3 *q*	2.17 *br s*	18.5 *q*	2.63 *br s*	17.8 *q*
10	2.75 *br s*	18.6 *q*	2.94 *br s*	18.8 *q*	2.79 *br s*	17.4 *q*
1′	5.11 *d* (6.9)	101.7 *d*	5.02 *d* (7.2)	102.3 *d*	—	—
2′	3.34 obs.	82.5 *d*	3.36 *dd* (9.4, 7.3)	83.4 *d*	—	—
3′	3.69 *m*	75.0 *d*	3.69 *dd* (9.4, 8.9)	76.2 *d*	—	—
4′	3.43 obs.	78.9 *d*	3.20 *m*	79.4 *d*	—	—
5′	a4.17 *m*	63.4 *t*	a3.83 *dd* (11.5, 5.0)	63.7 *t*	—	—
	b3.38 obs.		b3.11 *m*			
2′ OCH_3	3.72 *s*	60.7 *q*	3.57 *s*	60.5 *q*	—	—
4′ OCH_3	3.53 *s*	58.6 *q*	3.17 *s*	58.4 *q*	—	—

* obs. indicates signals obscured by overlapping resonances.

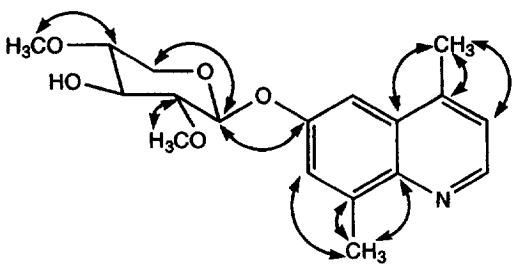
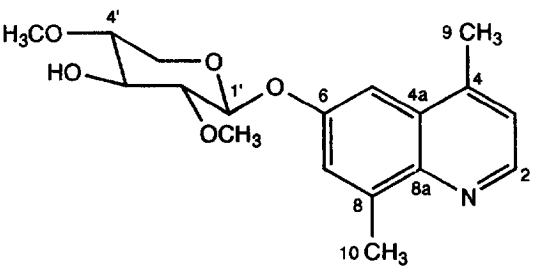


Fig. 1. HMBC correlations observed for 4,8-dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline (**1**).

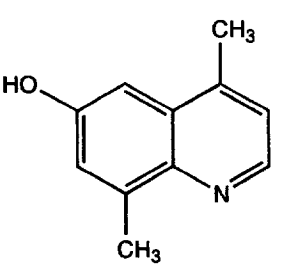
pound **1**, except for two methoxy groups (δ 3.57, 3H; δ 3.17, 3H).

The above structural deductions were verified by interpretation of long range correlations observed in the HMBC spectrum (J = 7 Hz) of 1 (Fig. 1). This experiment also enabled the location of the methoxy groups. The correlation cross-peaks observed from H_3-9 to C-3, C-4, and C-4a, as well as from H_3-10 to C-7, C-8, and C-8a confirmed their location at C-4 and C-8, respectively. The correlation from H_a-5′ and H_b-5′ to C-1′ confirmed the six-membered xylopyranose moiety. The cross-peak from the anomeric proton H-1′ to C-6 established the connectivity between the hydroxyquinoline and the xylopyranose moieties. The connections of the methoxy groups at C-2′ and C-4′ were established by the correlations from H_3 (δ 3.57) to C-2′ and H_3 (δ 3.17) to C-4′, respectively.

The coupling constant of 7.2 Hz for the anomeric proton (H-1′) indicated a β glycoside linkage. The coupling constants of 9.4 and 8.9 Hz between H-2′ and H-3′ and H-3′ and H-4′ suggested both to have *trans* diaxial relationships as expected in the xylopyranose unit [5]. The absolute configuration of the monosaccharide unit is proposed as D on the basis of its optical rotation ($[\alpha]_D^{25}$: −56.7°, c 0.15, $CHCl_3$). This sign and magnitude are both in agreement with the reference values found for phenyl-β-D-xylopyranoside ($[\alpha]_D^{20}$: −49.4°, c 1, H_2O) and methyl-2,4-di-*O*-methyl-β-D-xylopyranoside ($[\alpha]_D^{25}$: −79.0°, c 1, $CHCl_3$) [6, 7]. Thus, the complete structure of compound **1** was determined to be 4,8-dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline.



1



2

Compound **2** was obtained in very low yield as a white amorphous powder (0.6 mg, 0.02% of extract). The ^1H and ^{13}C NMR spectra of compound **2** showed it to possess the same 6-hydroxyquinoline moiety as found in **1** (see Table 1). However, compound **2** was lacking resonances for a xylose moiety. From these data, it was apparent that compound **2** was the aglycone of **1**. This was confirmed by EI-mass spectrometry data which showed a $[M]^+$ peak at *m/z* 173. Thus, compound **2** was defined as the new alkaloid 4,8-dimethyl-6-hydroxyquinoline (**2**). We have no specific data on whether compound **2** is a true natural product of *L. majuscula* or if it is formed artifactually from **1** by glycoside hydrolysis during extraction and isolation.

This is the first report of a hydroxyquinoline alkaloid in a marine cyanobacterium. In general, quinoline alkaloids are relatively rare in marine organisms, and are most commonly associated with sponge metabolism [8]. On the other hand, the 2,4-di-*O*-methyl-β-D-xylose moiety has been found in other marine organisms, especially as part of the saccharide chain of polyhydroxysterols from starfish [9]. Overall, our finding of five distinct classes of structurally novel natural products further attests to the spectacular biosynthetic capabilities of cyanobacteria in general [10], and in this collection of *L. majuscula* in particular.

EXPERIMENTAL

General. NMR spectra were recorded on a Bruker AM 400 spectrometer with the solvent used as an internal standard ($CDCl_3$ at δ 7.26 and 77.0; C_6D_6 at δ 7.16 and 128.0; CD_3OD at δ 3.31 and 49.0). MS: recorded on a Varian MAT 311 mass spectrometer. UV and IR spectra: recorded on Hewlett–Packard 8452A UV-VIS and Nicolet 510 spectrophotometers, respectively. OR: Perkin–Elmer model 141 polarimeter. TLC: Merck aluminium-backed. All solvents were distilled from glass prior to use.

Collection. The marine cyanobacterium *Lyngbya majuscula* (voucher specimen available from WHG as collection number NSB-15 Dec 92-2) was collected by hand from shallow water (0.1–1.0 m) on December 15 1991, at Barbara Beach (Spanish Waters), Curaçao, Netherlands Antilles, and stored in 2-propanol at red. temp. until work-up.

Extraction and isolation. A total of 295 g (dry wt) of the alga was extracted with CH_2Cl_2–MeOH (2:1, 2×) to give the crude extract (3.3 g). A portion of the crude extract (3.0 g) was fractionated using vacuum liquid chromatography (VLC) on silica gel with a stepwise gradient of hexane–EtOAc and EtOAc–MeOH. Eluted material was collected in fifteen 200 ml frs and monitored by TLC. Similar frs were combined to give eight frs. Fr. 7 (100 mg, eluted with 100% EtOAc) showed an ichthyotoxic effect and was further fractionated by column chromatography on Sephadex LH20 using EtOAc–MeOH (1:1) as eluent to give 18 × 10 ml frs. Similar frs were combined to yield five frs. Fr. 4 (35 mg) from the Sephadex column was further purified by flash chromatography on reversed phase (RP18) material using MeOH–H_2O 4:1 as an eluent. The final purification by prep. TLC (EtOAc–hexane, 4:1) yielded 4,8-dimethyl-6-*O*-(2′,4′-di-*O*-methyl-β-D-xylopyranosyl)-hydroxyquinoline (**1**, 1.5 mg).

Fr. 5 (258 mg, eluted with 50% EtOAc–hexane) showed a molluscicidal effect. After confirming (^1H NMR and molluscicidal effect) that the bioactive component barbamide was unreactive to CH_2N_2 (Et_2O, R_t, 5 min), the fr. was methylated and further fractionated by VLC on reverse phase (RP18) material using a MeOH–H_2O gradient (60% MeOH–100% MeOH). Methylation facilitated the chromatographic removal of contaminating fatty acids in this fr. as their methyl ester derivatives. Fr. 1 eluted with 60% MeOH, showed no molluscicidal effect, and was further purified by prep. TLC ($CHCl_3$–MeOH, 95:5) to yield 4,8-dimethyl-6-hydroxyquinoline, **2**, 0.6 mg.

4,8-*Dimethyl*-6-O-(2′,4′-*di*-O-*methyl*-β-D-xylopyranosyl)-hydroxyquinoline (**1**). White, amorphous. $[\alpha]_D^{25}$: −56.7° ($CHCl_3$, *c* 0.15); UV λ_{max}^{MeOH} 236 (log ε 4.65), 286 (3.57), 316 (3.44), 328 (3.44) nm; IR ν_{max} (film) cm^{-1}: 3602, 3008, 3000, 2892, 1620, 1507, 1464, 1208, 1111, 1093; HR-EIMS *m/z* 333.15780 ($[M]^+$, $C_{18}H_{23}NO_5$, calc. 333.15762); EIMS *m/z* (rel. int.): 333 $[M]^+$ (7), 173 (51), 160 (100), 144 (9), 101 (10). ^1H and ^{13}C NMR: see Table 1.

4,8-*Dimethyl*-6-*hydroxyquinoline* (**2**). White, amorphous. UV λ_{max}^{MeOH} 234 (log ε = 4.47), 283 (3.51), 322 (3.28), 333 (3.28) nm; IR ν_{max} (film) cm^{-1}: 3440, 3010, 1613, 1495, 1230, 1061; EIMS *m/z* (rel. int.): 173 $[M]^+$ (100), 158 (11), 144 (31). ^1H and ^{13}C NMR: see Table 1.

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REFERENCES

1. Orjala, J., Nagle, D. G., Hsu, V. L. and Gerwick, W. H., *Journal of the American Chemical Society*, 1995, **117**, 8281.
2. Orjala, J., Nagle, D. G. and Gerwick, W. H., *Journal of Natural Products*, 1995, **58**, 764.
3. Gerwick, W. H., Proteau, P. J., Nagle, D. G., Hamel, E., Blokhin, A. and Slate, D. L., *Journal of Organic Chemistry*, 1994, **59**, 1243.
4. Orjala, J. and Gerwick, W. H., *Journal of Natural Products*, 1996, **59**, 427.
5. Agrawal, P. K., *Phytochemistry*, 1992, **31**, 3307.

6. Montgomery, E. M., Richtmeyer, N. K. and Hudson, C. S., *Journal of the American Chemical Society*, 1942, **64**, 690.
7. Ferrier, R. J., Prasad, D., Rudowski, A. and Sangster, I., *Journal of the Chemical Society*, 1964, 3330.
8. Sun, H. H., Sakemi, S., Burres, N., McCarthy, P., *Journal of Organic Chemistry*, 1990, **55**, 4964.
9. D'Auria, M. V., Minale, L. and Riccio, R., *Chemistry Review*, 1993, **93**, 1839.
10. Moore, R. E., *Journal of Industrial Microbiology*, 1996, **16**, 134.