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ISOLATION OF A CYCLIC CARBONATE, A γ-BUTYROLACTONE, AND A NEW INDOLE DERIVATIVE FROM THE MARINE CYANOBACTERIUM *LYNGBYA MAJUSCULA*

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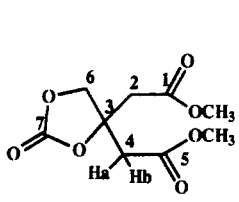
ABSTRACT.—Two new citric acid derivatives, a cyclic carbonate and a γ-lactone, and a new *N*,7-dimethylindole-3-carboxaldehyde, have been isolated from the marine cyanobacterium *Lyngbya majuscula*.

Long known as a rich source of novel natural products, the marine cyanobacterium *Lyngbya majuscula* Harvey ex Gomont (Oscillatoriaceae) (1) continues to yield new structural classes of compounds, such as curacin A (2). This note further illustrates the organism's ability to produce diverse compounds by describing the isolation of a unique cyclic carbonate [**1**] and its possible lactone precursor [**2**], a new indole derivative [**3**], and a metabolite previously known from a red alga [**4**], all from a shallow water Okinawan collection of *L. majuscula*. These compounds were found in chromatographic fractions showing brine shrimp toxicity (3) that was most likely caused by debromoaplysiatoxin, the major component of the mixture (4).



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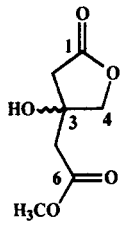

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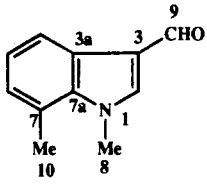

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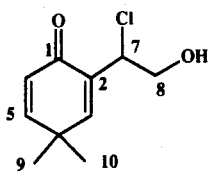

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The structure of lyngbyacarbonate [**1**] was elucidated by nmr, ms, and ir data. Integration of the peaks in the simple ^{1}H-nmr spectrum, together with DEPT-135 data, indicated a symmetrical molecule with three isolated spin-systems. Although the long relaxation time of C-7 initially prevented detection of this peak in the ^{13}C-nmr spectrum of **1**, recording of its spectrum with a relaxation delay of 5 sec revealed the position of this hidden peak at δ 153.6, indicative of a cyclic carbonate. Moreover, the molecular formula from positive-ion hrfabms and the ir band at 1805 cm^{-1} further substantiated the presence of this unusual functionality. HMBC nmr data provided final confirmation of structure **1**.

Lyngbyacarbonate [**1**] represents a new structural class of marine natural product. The only other naturally occurring cyclic carbonates have been found in filamentous bacteria. *Streptomyces* spp. produce aldgarose as the glycoside of the antibiotic aldgamycin E (*S. lavendulae*) (5) and the antibiotic neocarzinostatin chromophore (*S. carzinostaticus*) (6). In addition, an antifungal triynecarbonate has been isolated from *Actinomycetes* spp. fermentation broths (7). However, no biological activity has been associated with the cyclic carbonate structure to date. Lyngbyacarbonate is not cytotoxic against HCT-116 and KB cancer cell lines,[2] and the DNA strand-scission ac-

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[2]Bioassays provided by Dr. Louis R. Barrow, Department of Pharmacology, University of Utah.

tivity in neocarzinostatin chromophore is unaffected by the absence of the carbonate moiety (8).

Characterization of γ-lactone **2** (hrcims molecular formula $C_7H_{11}O_5$) was accomplished by essentially the same strategy as used above for lyngbyacarbonate [**1**]. The broad ir band at 3455 cm^{-1} indicated a hydroxyl group while bands at 1783 and 1727 cm^{-1} suggested the presence of γ-lactone and ester moieties, respectively. Additional data from ^1H-, ^{13}C-, and DEPT-135 nmr spectra showed the presence of three quaternary carbon atoms, three isolated CH_2's, and a OMe group. These structural fragments could be assembled into only a single planar structure, yielding γ-lactone **2**. The absolute configuration of **2**, $[\alpha]^{24}D$ $-3.0°$ (c=0.23, $CHCl_3$), remains unknown.

Although structurally similar to the known cyanobacterium metabolite 3-hydroxybutyrolactone (9), compound **2** may also be considered as a lactone derivative of citric acid in which the central carboxyl group has been reduced to an alcohol. The open form of the lactone **2** could serve as precursor to lyngbyacarbonate [**1**] by reaction with bicarbonate in a manner analogous to the mechanism established for aldgarose (10).

Nmr experiments were used for the structure determination of indole derivative **3** ($C_{11}H_{11}NO$). The chemical shifts from the ^1H-nmr spectrum indicated the presence of an aldehyde group, two methyl groups, and four aromatic protons, while the spin-coupling patterns suggested a trisubstituted indole system with three adjacent protons. The four quaternary carbons detected by ^{13}C-nmr and DEPT-135 data were consistent with an *N*-methylindole derivative. Positional assignments for the aldehyde and methyl groups were based on HMBC cross-peaks between C-7a and H-8, -10; C-6 and H-10; C-2 and H-8; and C-3a and H-9. Verification was provided by NOESY cross-peaks between H-6 and H-10 and between H-2 and H-8. The highfield shift for H-8 (δ 4.13) was attributed to conjugation effects between the nitrogen and aldehyde groups. These data, plus the hrcims molecular formula, established compound **3** as the new indole derivative *N*,7-dimethylindole-3-carboxaldehyde.

Metabolite **3** is unusual in several respects. Although indole-3-carboxaldehyde has been isolated from the red alga *Botrocladia leptopoda* (11), **3** is the first aldehyde of any kind to be found in a cyanobacterium (1,12,13). Furthermore, *N*-methylindoles are uncommon in algae, the only other examples being three bromo indoles isolated from the red alga *Laurencia brongniartii* (12,14). Finally, no other 7-methylindoles have been found in marine algae. Apparently compound **3** is a widespread minor metabolite of *L. majuscula*, as it has subsequently been detected in another collection of this alga from Curaçao (private communication from Dr. Jimmy Orjala, College of Pharmacy, Oregon State University).

The structure of monoterpene **4** was elucidated by ^1H-nmr, ^{13}C-nmr, DEPT-135, ^1H-^1H COSY, ^{13}C-^1H COSY, and HMBC experiments and was found to be identical to a compound isolated by Higa from the red alga *Desmia hornemanni* (=*Portieria hornemanni*) (15). Although the investigated sample of *L. majuscula* was visually homogeneous, the relatively low concentration of metabolite **4**, as well as of metabolites **1–3**, raises a question as to the true origin of these compounds. The occurrence of monoterpene **4** in *L. majuscula* is unusual since cyanobacteria are not generally known as a source of these compounds except as presumed subunits in larger molecules, such as lyngbyatoxin (16). To our knowledge, β-cyclocitral is the only other monoterpene known from a cyanobacterium (*Microcystis wesenbergii*) (13).

The biological function, if any, of compounds **1–4** is presently unknown. The apparent rarity of cyclic carbonates in nature poses questions concerning their

biosynthesis. In the case of aldgarose, radioactive labeling experiments indicate that this functionality arises from a carboxylation reaction involving bicarbonate ion (10). If a similar mechanism applies to lyngbyacarbonate, then an unusual reduced form of citric acid, perhaps related to γ-lactone **2**, would most likely be involved. As cyanobacteria have been shown to possess an incomplete citric acid cycle unable to transform α-ketoglutarate to succinyl-CoA (17), it is tempting to speculate that metabolites **1** and **2** represent shunt metabolites of citrate.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr experiments were done in $CDCl_3$ on Bruker AC-300 and AM-400 spectrometers using solvent as reference (δ 77.0 ppm) for ^{13}C and TMS for ^1H spectra. Coupling constants are given in Hz.

PLANT MATERIAL.—*Lyngbya majuscula* (582 g wet wt, 81 g dry wt) was collected on a reef just offshore from Uken, Okinawa, Japan, in July 1993. A voucher specimen has been deposited at the College of Pharmacy, Oregon State University.

EXTRACTION AND ISOLATION.—The alga was stored frozen in MeOH prior to extraction with MeOH-CH_2Cl_2 (1:2, 3×250 ml) at room temperature. After removal of H_2O by partition, the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to yield a green oil (2.80 g) that was chromatographed on a Si gel column (55×30 mm i.d., vlc) using an EtOAc/hexanes gradient. The residue (1067 mg) from a brine shrimp toxic fraction (50% EtOAc/hexanes) was purified on a reversed-phase column (Bakerbond C_{18} ODS, 20×22 mm i.d., 80% MeOH/H_2O) which separated debromoaplysiatoxin (216 mg) from a mixture (30 mg) of **1**, **3**, and **4**. Purification of the mixture by normal-phase hplc (Phenomenex Maxsil 10 Si gel, 500×10 mm i.d., 50% EtOAc/hexanes) and reversed-phase hplc (Hibar Li Chrosorb RP-18, 7 μm, 250×10 mm i.d.; 60% MeOH/H_2O) gave **1** (5 mg, white needles), **3** (1 mg, white solid), and **4** (10 mg, colorless oil). Compound **2** (3 mg, colorless oil) was isolated from another vlc fraction (129 mg, 100% EtOAc) by use of a reversed-phase column (*vide supra*) prior to purification by normal-phase hplc (*vide supra*, 60% EtOAc/hexanes).

Lyngbyacarbonate [**1**].—Ft-ir (film) ν max 2959, 1805, 1733, 1438, 1363, 1198, 1065, 963, 764 cm^{-1}; ^1H nmr (400 MHz) δ 4.53 (2H, s, H-6), 3.73 (6H, s, 2×OMe), 3.10 (2H, d, J=16.7 Hz, H-2a, -4a), 2.94 (2H, d, J=16.7 Hz, H-2b, -4b); ^{13}C nmr, DEPT-135, and C-H COSY (100 MHz) δ 169.0 (2C, s, C-1, -5), 153.5 (s, C-7), 79.4 (s, C-3), 72.8 (t, C-6), 52.3 (2C, q, 2×OMe), 41.5 (2C, t, C-2, -4); HMBC cross-peaks (C-1, -5, and OMe), (C-2, -4, and H-6), (C-3 and H-6, -2ab, -4ab), (C-7 and H-6), (C-6 and H-2ab, -4ab); hrfabms (positive-ion) (3-nitrobenzyl alcohol matrix) *m/z* $[M+1]^+$ 233.06620, $C_9H_{13}O_7$ (Δ 0.1 mamu dev.).

γ-Lactone **2**.—Ft-ir (film) ν max 3455, 1783, 1727, 1440, 1367, 1209, 1175, 1024 cm^{-1}; ^1H nmr (300 MHz, * assignments interchangeable) δ 4.39 (1H, d, J=9.9 Hz, H-4), 4.16 (1H, d, J=10.0 Hz, H-4), 3.77 (4H, br s, OCH_3, OH), 2.83 (1H, d, J=17.0 Hz, H-2*), 2.76 (1H, d, J=17.6 Hz, H-5*), 2.74 (1H, d, J=17.0 Hz, H-2*), 2.56 (1H, d, J=17.6 Hz, H-5*); ^{13}C nmr, DEPT-135, and C-H COSY (75.5 MHz, * or $^+$ assignments interchangeable) δ 174.4 (s, C-1*), 171.9 (s, C-6*), 77.8 (t, C-4), 74.2 (s, C-3), 52.4 (q, C-7), 41.9 (t, C-5$^+$), 41.4 (t, C-2$^+$); hrcims (CH_4) *m/z* $[M+1]^+$ 175.06060, $C_7H_{11}O_5$ (Δ 0.0 mamu dev.).

N-7-Dimethylindole-3-carboxaldehyde [**3**].—Ft-ir (film) ν max 3114, 2930, 1652, 1542, 1459, 1416, 1385, 1102, 779, 744 cm^{-1}; uv (EtOH) λ max 216 (ε 22000), 246 (ε 13000), 306 (ε 12000) nm; ^1H nmr (300 MHz) δ 9.98 (1H, s, H-9), 8.17 (1H, d, J=7.8 Hz, H-4), 7.57 (1H, s, H-2), 7.17 (1H, dd, J=7.5 and 7.7 Hz, H-5), 7.04 (1H, d, J=7.2 Hz, H-6), 4.13 (3H, s, CH_3-8), 2.77 (3H, s, CH_3-10); ^{13}C nmr, DEPT-135, and C-H COSY (100 MHz) δ 184.3 (d, C-9), 140.6 (d, C-2), 136.6 (s, C-7a), 126.8 (d, C-6), 126.5 (s, C-3a), 123.1 (d, C-5), 121.7 (s, C-7), 120.1 (d, C-4), 117.7 (s, C-3), 37.9 (q, C-8), 19.5 (q, C-10); HMBC cross-peaks (C-2 and H-8), (C-3 and H-2, -9), (C-4 and H-6), (C-6 or -3a and H-2, -4, -5, -9, -10), (C-7 and H-5, -10), (C-7a and H-2, -4, -6, -8, -10), (C-10 and H-6); gc-ms (70 ev) *m/z* 173 (76.9), 172 (100), 144 (16.1); hrcims (CH_4) *m/z* $[M+1]^+$ 174.09180, $C_{11}H_{12}NO$ (Δ −0.1 mamu dev.).

Monoterpene **4**.—$[\alpha]^{28}D$ −65.4° (c=0.49, CH_2Cl_2); Ft-ir (film) ν max 3421, 2970, 2930, 1664, 1630 cm^{-1}; uv (EtOH) λ max 238 (ε 13000) nm; ^1H nmr (300 MHz) essentially the same as the literature (13); ^{13}C nmr, DEPT-135, H-H COSY, C-H COSY (100 MHz, *assignments differ from literature) δ 183.8 (s, C-1), 156.7 (d, C-5*), 155.3 (d, C-3*), 133.9 (s, C-2), 126.9 (d, C-6), 66.4 (t, C-8*), 59.0 (d, C-7*), 38.3 (s, C-4), 26.7 (2C, q, C-9, -10); HMBC (mixture of **3** and **1**) cross-peaks (C-1 and H-3, -5, -6, -7), (C-2 and H-3, -6, -7), (C-3 and H-5, -7, -9), (C-4 and H-3, -5, -6, -9), (C-5 and H-3, -9), (C-7 and H-3, -6), (C-8 and H-7), (C-

9, -10 and H-3, -5); hrfabms (positive ion, (3-NBA matrix) *m/z* $[M+1]^+$ 201.06810, $C_{10}H_{14}ClO_2$ (Δ −0.1 mamu dev.).

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