LOULUDINIUM CHLORIDE, AN AZABICYCLONONANE ALKALOID FROM THE MARINE CYANOPHYTE *LYNGBYA GRACILIS*

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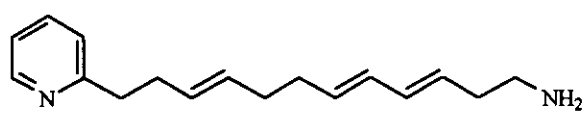
Abstract- A blue-green alga, *Lyngbya gracilis*, which was collected in the lagoon of Palmyra island, yielded a single metabolite, louludinium chloride (**4**). It is a rarely encountered azabicyclo[3.3.0]nonane derivative with moderate activity in the KB assay.

Marine invertebrates, notably mollusks and sponges, have given rise to relatively simple as well as highly complex alkylpyridines. Among our recently studied examples are the α-substitued naloamine (**1**) from a herbivorous mollusk,[1] the β-alkyl-substituted ikimines (**2**) from a sponge,[2] and a manzamine dimer kauluamine (**3**), also from a sponge.[3]



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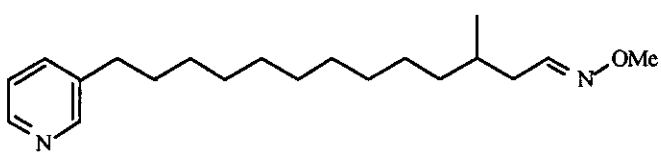

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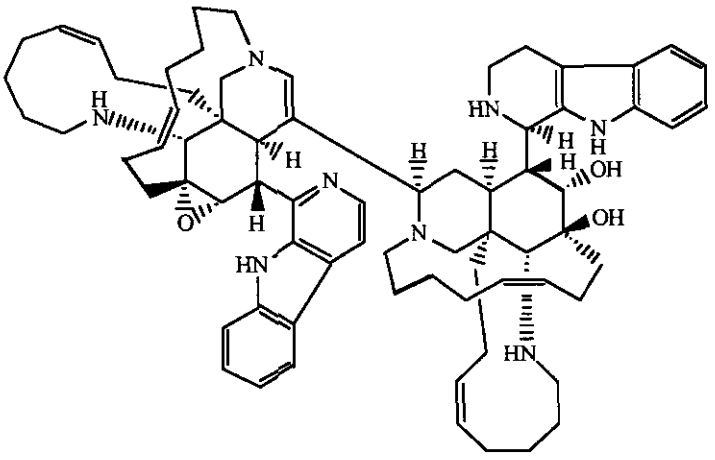

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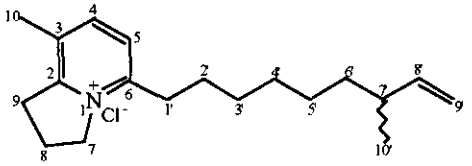
In this report we describe isolation and structure of louludinium[4] chloride (**4**) from a marine bluegreen alga, *Lyngbya gracilis*.



4

The alga was collected in 1989 at -10 m in the Palmyra atoll lagoon (5°53'N, 162°5'W), frozen, and subsequently extracted with ethanol. The ethanolic concentrate was partitioned against hexane, chloroform, and finally *n*-butanol. The chloroform fraction was purified by flash chromatography and crystallized from chloroform to yield louludinium chloride (**4**) as colorless crystals, mp >300° (decomp).

UV (λ_{max} 278 nm) and IR (1625 cm^{-1}) spectral data indicated the presence of a pyridinium ion on the basis of pertinent literature values.[5-7] A molecular formula of $C_{19}H_{30}N$ for **4** was based on a positive ion HRFABMS determination[8] (M^+ 272.2382 Da; calcd 272.2378, glycerol matrix). These data required an ionic compound with 5.5 rings and/or double bonds. Prominent signals in the ^1H NMR spectrum, doublets at δ 8.23 (H4) and 7.71 (H5) coupled with J=7.9 Hz, defined a trisubstituted pyridinium salt.[6,7] The three alkyl substituents appeared to be a 3H singlet at δ 2.39 (Me10), a 2H triplet (J =7.7 Hz) at δ 2.97 ($H_2$1'), and another 2H triplet (J=7.8 Hz) at δ 3.41 ($H_2$9). A COSY experiment showed that this signal at δ 3.41 was coupled to a 2H quintet at δ 2.36 (J=7.8 Hz, H8), which in turn was coupled to

triplet (J=7.8 Hz) at δ 4.77 ($H_2$7) characteristic of a methylene vicinal to a quaternary nitrogen,[5-7] thereby defining a fused azabicyclononane ring system. COSY experiments also defined a C_{10} chain beginning with the 2H triplet at δ 2.97 ($H_2$1') and terminating in an olefin which was defined by a ^{1}H resonance at δ 5.68 (H8') and two mutually coupled signals at δ 4.94 and 4.89 ($H_2$9').

Table 1. NMR Data for 4, Measured in DMSO-d_6

C#	^{13}C	^{1}H, mult, J (Hz)	HMBC
2	157.4		
3	131.6		
4	144.7	8.23, 1H, d (7.9)	C2, C6, C10
5	124.5	7.71, 1H, d (7.9)	C3, C6, C1'
6	152.7		
7	56.5	4.77, 2H, t, (7.8)	C2, C6, C8, C9
8	19.8	2.36, 2H, quint (7.8)	C2, C7, C9
9	30.9	3.41, 2H, t (7.8)	C2, C7, C8
10	17.0	2.39, 3H, s	C2, C3, C4
1'	31.6	2.97, 2H, t (7.7)	C5, C6, C2', C3'
2'	26.4	1.67, 2H, quint (7.7)	C6, C1', C3'
3'	28.3	1.37, 2H, m	C2', C4'
4'	28.6	1.31, 2H, m	C5'
5'	26.3	1.26, 2H, m	
6'	35.8	1.25, 2H, m	C5', C8'
7'	36.8	2.08, 2H, brm	C6'
8'	144.4	5.68, 1H, ddd	C7'
		(17.3, 10.2, 7.4)	
9'	112.5	4.94, 1H, ddd	C7', C8'
		(17.3, 2.0, 1.1)	
		4.89, 1H, ddd	C7'
		(10.2, 2.0, 1.1)	
10'	19.7	0.93, 3H, d (6.7)	C6', C7', C8'

The ^{13}C NMR spectrum displayed 19 carbon resonances, twelve sp^3 and seven sp^2 carbons. According to a DEPT experiment there were two methyl groups, ten methylenes, four methines, and two quaternary carbons. Carbon-hydrogen connectivities were determined by an HMQC experiment.[9] Isolated spin systems were assigned by COSY experiments. HMBC experiments[10] served to delineate the skeleton. Details are recorded in Table 1. The azabicyclo[3.3.0]nonane structure was supported by correlations from H7 to C2 and C6 and by cross-peaks from H8 and H9 to C2. Additional correlations were seen between H10 and C2, C3, and C4 and from H1' to C5 and C6.

Louludinium chloride (**4**) displays a structure which had not previously been seen among marine natural products. As has been pointed out, alkylpyridines and their salts are encountered frequently,[1-3,5-7] but the azabicyclo[3.3.0]nonane system has been encountered as a structural feature of constituents of some dendrobatid poison frogs.[11]

In the KB (human oral epidermoid carcinoma) assay louludinium chloride (**4**) was active with an MIC of 5 μg/mL.

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EXPERIMENTAL

NMR spectra were obtained on General Electric QE-300 and GN Omega 500 instruments. MS spectra were obtained with VG 70/SE and JEOL JMX SX102 mass spectrometers. IR spectra were measured on a Perkin-Elmer 1600 spectrometer. UV spectra were determined on a Hewlett Packard 8452A diode array spectrophotometer. Optical rotations were taken on a Jasco DIP-370 digital polarimeter. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. All solvents were distilled prior to use. *Lyngbya gracilis* was collected at -10 m in the Palmyra atoll lagoon in 1989. The frozen sample (125 g)

was extracted with 3x 250 mL EtOH and the combined ethanolic extracts were concentrated and successively partitioned against hexane, chloroform and *n*-BuOH. The chloroform partition was concentrated *in vacuo* to yield 200 mg of a dark green solid. The solid was subjected to cyano flash chromatography with 100% MeOH to yield a substance that was 90% pure; it was further purified by crystallization from $CHCl_3$ to afford 21 mg of **4**.

Louludinium chloride (**4**).- Colorless crystals, mp > 300° (decomp); $[\alpha]_D^{23}$ +97° (*c* 0.65, MeOH); positive ion HRFABMS obs'd *m/z* 272.2382, $C_{19}H_{30}N$ requires 272.2378; IR (neat) 3400, 2930, 2855, 2085, 1625, 1510, 1460, 1170, 910; UV (MeOH) 278 (ε 800), 218 (ε 1200), 204 (ε 1700); ^{13}C and ^{1}H NMR, see Table 1.

REFERENCES AND NOTES

1. C. M. Szabo, Y. Nakao, W. Y. Yoshida, and P. J. Scheuer, *Tetrahedron*, *1996*, **52**, 9681.
2. A. R. Carroll and P. J. Scheuer, *Tetrahedron*, *1990*, **46**, 6637.
3. I. I. Ohtani, T. Ichiba, M. Isobe, M. Kelly-Borges, and P. J. Scheuer, *J. Am. Chem. Soc.*, *1995*, **117**, 10743.
4. *Loulu* is the Hawaiian name of the fan palm known as Palmyra, which is also the island, where the organism was collected.
5. F. J. Schmitz, K. H. Hollenbeak, and D. C. Campbell, *J. Org. Chem.*, *1978*, **43**, 3916.
6. R. Talpir, A. Rudi, M. Ilan, and Y. Kashman, *Tetrahedron Lett.*, *1992*, **33**, 3033.
7. M. T. Davies-Coleman, J. D. Faulkner, G. M. Dubowchik, G. P. Roth, C. Polson, and C. Fairchild, *J. Org. Chem.*, *1993*, **58**, 5925.
8. In the negative ion FABMS of **4** peaks of *m/z* 35 (Cl) and 127 (Cl + glycerol) were observed, thus establishing chloride as the counter-ion.
9. A. Bax and S. Subramanian, *J. Magn. Reson.*, *1986*, **67**, 565.
10. A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, *1986*, **108**, 2093.
11. P. Jain, H. M. Garraffa, T. F. Spande, H. J. C. Yeh, and J. W. Daly, *J. Nat. Prod.*, *1995*, **58**, 100.

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