potassium part of the solution (background) compared with the potassium contribution. In fact, the slight difference between $I$ and $G$ or $H$ in the peak region and in the height of the shoulder compared to that of the peak is mainly due to this difference in background. Thus spectra $G$, $H$, and $I$ provide a measure of the uniqueness of the purely aqueous environment. The near-edge spectrum of hydrated potassium is insensitive to both counterion and potassium concentration.

This uniqueness makes it meaningful to compare the spectrum of the cells with that of the aqueous solution. Figure 2 shows magnified spectra in the peak region, 3608 to 3626 eV. In the blood sample, more than 95 percent of the potassium ions are intracellular. The intracellular potassium concentration is between 0.1 and 0.2$M$. Spectrum $A$ in Fig. 2 is the peak region of $J$ in Fig. 1. The measuring time for each data point on $J$ in Fig. 1 was 10 seconds. Spectra $B$ and $C$ in Fig. 2 are the same spectrum with better statistics—30 and 50 seconds per point, respectively. One can see that certain features of the potassium spectrum of blood cells are not statistical fluctuations, and that they are qualitatively different from the features of the spectrum of free potassium at a similar concentration, $D$ in Fig. 2. In particular, the height of the low-energy peak at ~ 3610 eV relative to the broad second peak is quite different between the two. The broad peak of the blood cells is shifted to lower energy and is broader, possibly indicating a greater heterogeneity of the $K^+$ environment than in $H_2O$. Because the $K^+$ environments we examined appear fairly unique and distinguishable from one another, we conclude that the differences represented in Fig. 2 are characteristic of the complex binding of $K^+$ in these frog blood cells and that, for potassium, the cell cannot be regarded as an aqueous solution.

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6. The monochromator was calibrated at the Cu edge (8980 eV). At the potassium edge the scale could be off by a few electron volts.
7. We have tentatively assigned the first peak to the 4*s* vibronic state, and the second peak to the allowed 4*p* state.
8. Stability constants for valinomycin complexes were from W. Simon, W. E. Morf, P. Ch. Meier, *Struct. Bonding (Berlin)* **16**, 114 (1973). Solubilities of $K^+$ were from H. Stephen and T. Stephen, Eds., *Solubilities of Inorganic and Organic Compounds* (Macmillan, New York, 1963).
9. We thank G. N. Ling for the blood cell samples and SSRL for their hospitality and assistance. Supported in part by ONR contracts N00014-77-C-0239 and N00014-76-C-0273 (to Rice University), PHS grant GM 21721 from the National Institute of General Medical Sciences (Rice University), and the Robert A. Welch Foundation (Rice University and the University of Houston).

8 January 1979

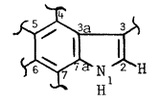
## Seaweed Dermatitis: Structure of Lyngbyatoxin A

Abstract. *A highly inflammatory and vesicatory substance, lyngbyatoxin A, has been isolated from the lipid extract of a Hawaiian shallow-water variety of* Lyngbya majuscula *Gomont; its gross structure was determined from chemical and spectral data. Lyngbyatoxin A is closely related to teleocidin B, a poisonous substance associated with several strains of* Streptomyces.

The blue-green alga *Lyngbya majuscula* Gomont is responsible for a severe erythematous, papulovesicular dermatitis known as "swimmers' itch" in Hawaii (*1*, *2*). Since all subjects who come into direct contact with the noxious strains of this cyanophyte develop erythema, followed by blisters and deep desquamation, the active principle is a primary irritant and not an allergen (*2*, *3*). Debromoaplysiatoxin, a poisonous substance that is present (*4*) in a deep-water variety of *L. majuscula* from Enewetak (*5*) and in dermatitis-producing *L. majuscula* from Laie Bay, Oahu (*6*), produces an erythematous pustular folliculitis in humans (*7*) and could be the causative agent of the dermatitis. Not all strains of toxic *L. majuscula*, however, contain debromoaplysiatoxin or its congeners. For example, a toxic shallow-water variety of *L. majuscula* from Kahala Beach, Oahu, contains a different compound, lyngbyatoxin A, which is also a highly inflammatory and vesicatory agent. The structure determination of lyngbyatoxin A, which may represent the first indole alkaloid from a marine plant, is the subject of this report.

Freeze-dried *L. majuscula* from Kahala Beach, Oahu, was extracted successively with petroleum ether and dichloromethane. Gel filtration of the dichloromethane extract on Sephadex LH-20 (*8*) followed by high-pressure liquid chromatography of the toxic fraction on $\mu$-Bondapak-CN with a mixture of hexane and diisopropyl ether (1:1) gave lyngbyatoxin A as a tan gummy solid in 0.02 percent yield.

High-resolution mass spectrometry established the molecular formula of lyngbyatoxin A as $C_{27}H_{39}N_3O_2$ (found: *m/e* 437.3019) and the $^{13}C$ nuclear magnetic resonance (NMR) spectrum (Table 1) confirmed that 27 carbon atoms were present in the molecule. The ultraviolet spectrum of the toxin in ethanol, which showed bands at 212 ($\epsilon$ 18,200), 231 (26,300), 287 (8,900), and 301 nm (9,300), was typical of an indole. A positive Ehrlich test supported the presence of this moiety and also suggested that the C-2 position of the indole was unsubstituted. The $^1H$ NMR spectrum (Table 1) (*9*) indicated that the indole was trisubstituted as it exhibited a broad singlet at $\delta$ 8.50 for the indole NH, a broad singlet at $\delta$ 6.81 for the C-2 proton, and two sharp doublets ($J$ = 8 Hz) at $\delta$ 6.44 and 6.96 assigned to two vicinal protons on either C-4 and C-5, C-5 and C-6, or C-6 and C-7.




a

Further inspection of the $^1H$ NMR spectrum revealed doublets at $\delta$ 0.62 and 0.89 for secondary methyl groups, a doublet at $\delta$ 4.33 for a methine proton, a multiplet at $\delta$ 2.55 for a methine proton that was strongly coupled to all three signals at $\delta$ 0.62, 0.89, and 4.33, and a singlet at $\delta$ 2.87 for an *N*-methyl group of an *N*-methylvaline unit (**b**) (*10*). The amino acid, however, could not be liberated on vigorous acid hydrolysis. The carbonyl in **b** had to be connected to a nitrogen since the $^{13}C$ NMR spectrum had only one carbonyl carbon signal ($\delta$ 174.7) and the infrared spectrum indicated that it was in an amide group (1650 $cm^{-1}$). The N-13 nitrogen in **b** was therefore attached to the rest of the molecule by a bond that could not be cleaved by acid hydrolysis. This nitrogen also was very weakly basic as the toxin could not be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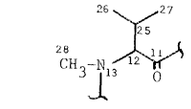
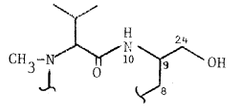
extracted from a chloroform solution into dilute aqueous hydrochloric acid.

Spin-spin decoupling experiments at 360 MHz allowed an expansion of partial structure **b** to **c**. The two doublets at



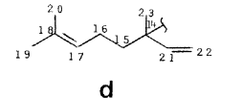
**b**



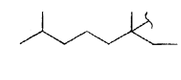
**c**

δ 3.56 and 3.72 were assigned to nonequivalent protons of a methylene (C-24) bearing methine and hydroxyl groups. Coupling between the C-24 protons and the hydroxyl proton was not observed. To prove that an OH group was attached to C-24, the toxin was treated with acetic anhydride and pyridine. As was expected, the resulting *O*-monoacetate exhibited the C-24 proton signals at δ 4.00 and 4.14. The two doublets of doublets at δ 3.05 and 3.11 were assigned to nonequivalent protons of a methylene (C-8) that was connected to the same methine (C-9) as the C-24 methylene. The large negative geminal coupling constant for the C-8 protons ($J = -17.5$ Hz) indicated that the C-8 methylene was attached to a doubly bonded carbon, which appeared to be C-3 since double resonance experiments showed a small coupling of the C-8 protons to the C-2 proton of the indole. The broad band at δ 4.32 was ascribed to the C-9 methine, and this proton also showed coupling to the amide NH proton (N-10) resonating at δ 7.77.

The $^{13}C$ NMR spectrum exhibited 12 olefinic carbon signals. Eight of these were attributed to the indole system, which meant that lyngbyatoxin A had to have two additional carbon-carbon double bonds (a singlet at δ 131.3, doublets at δ 148.3 and 124.4, and a triplet at δ 112.1). Catalytic hydrogenation (Pd on charcoals) of lyngbyatoxin A produced a tetrahydrotoxin (*11*) which had the same ultraviolet spectrum as that of the toxin. Both double bonds were therefore unconjugated. The presence of a vinyl group was recognized from the diagnostic signals at δ 5.26, 5.30, and 6.15 in the $^1H$ NMR spectrum and from bands at 915 and 990 $cm^{-1}$ in the infrared spectrum. The signal for the vinyl methine proton showed coupling to only the terminal methylene protons and thus the vinyl group had to be connected to the sole quaternary $sp^3$ carbon in the toxin ($^{13}C$ singlet at δ 43.0). The broad multiplet at δ 5.06 was attributed to the olefinic proton of a trisubstituted double bond and double-resonance experiments established that this portion was strongly coupled to nonequivalent protons of an adjacent methylene (broad multiplet at δ 1.70 and 1.93) and weakly coupled to two allylic methyl groups (broad singlets at δ 1.47 and 1.63). The resulting isopent-2-enyl group was attached to a nonequivalent methylene group (triplets of doublets at δ 1.80 and 1.89) which in turn was attached to the quaternary carbon along with the vinyl group and a methyl group (singlet at δ 1.44). A linalyl group (**d**) was therefore present in the toxin. In the $^1H$ NMR spectrum of the tetrahydrotoxin, the C-19 and C-20 methyl groups resonated as doublets ($J = 7$ Hz) at δ 0.74 and 0.76, which both collapsed simultaneously to singlets on irradiation of a $^1H$ multiplet (C-18) at δ 1.43, and the ethyl group resonated as a triplet at δ 0.63 ($J = 7$ Hz) for the methyl protons on C-22 and two doublets ($J_{gem} = -14$ Hz) of quartets ($J = 7$) for the nonequivalent methylene protons on C-21. The presence of **d** in the toxin was further supported by the mass spectrum which showed fragment ions at *m/e* 422 and 354 for loss of methyl and isohex-3-enyl groups from the molecular ion; loss of the vinyl group, however, was not observed. Similarly the presence of **e** in tetrahydrolyngbyatoxin A was supported by fragment ions at 426, 412, and 356 for loss of methyl, ethyl, and isohexyl groups from the molecular ion (*12*).



**d**



**e**

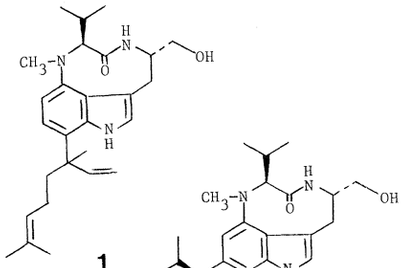
From these data we have concluded that lyngbyatoxin A has structure **1**. Placement of the linalyl group at C-7 rather than at C-5 is more compatible with the chemical shifts for the two vicinal aromatic protons (*8*). The gross structure is closely related to that of teleocidin B (**2**), a toxic substance found in the mycelia of several strains of *Streptomyces* (*13*). The stereochemistry and ab-

Table 1. Nuclear magnetic resonance data for lyngbyatoxin A (*8*). Abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; gem, geminal; and $J$, coupling constant.

| Carbon-13 chemical shift* | Assignment† | Proton chemical shift |
|---|---|---|
| 174.7 (s) | 11 | |
| 148.3 (d) | 21 | 6.15 (dd, $J_{21,22} = 18$, $J_{21,22'} = 10$ Hz) |
| 146.2 (s) | 4 | |
| 137.2 (s) | 7a | |
| 131.3 (s) | 18 | |
| 124.4 (d) | 17 | 5.06 (br, m) |
| 121.4 (s) | 3, 3a, or 7 | |
| 120.7 (d) | 2 | 6.81 (br, s) |
| 119.7 (d) | 6 | 6.96 (d, $J_{5,6} = 8$ Hz) |
| 118.4 (s) | 3, 3a, or 7 | |
| 114.1 (s) | 3, 3a, or 7 | |
| 112.1 (t) | 22 | 5.30 (unresolved dd‡, $J_{21,22} = 18$ Hz) |
| | | 5.26 (unresolved dd, $J_{21,22'} = 10$ Hz) |
| 106.1 (d) | 5 | 6.44 (d, $J_{5,6} = 8$ Hz) |
| 70.6 (d) | 12 | 4.33 (d, $J_{12,25} = 12$ Hz) |
| 64.7 (t) | 24 | 3.72 (dd, $J_{gem} = -11.5$, $J_{9,24} = 3$ Hz) |
| | | 3.56 (dd, $J_{gem} = -11.5$, $J_{9,24'} = 8.5$ Hz) |
| 55.7 (d) | 9 | 4.32 (br, s) |
| 43.0 (s) | 14 | |
| 38.3 (t) | 15 | 1.89 (td, $J_{gem} = -12$, $J_{15,16} = 12$, $J_{15',16} = 3$ Hz) |
| | | 1.80 (td, $J_{gem} = -12$, $J_{15,16'} = 12$, $J_{15',16'} = 3$ Hz) |
| 33.7 (t) | 8 | 3.11 (dd, $J_{gem} = -17.5$, $J_{8,9} = 2$ Hz) |
| | | 3.05 (dd, $J_{gem} = -17.5$, $J_{8',9} = 3.5$ Hz) |
| 32.9 (q) | 28 | 2.87 (s) |
| 28.1 (d) | 25 | 2.55 (m) |
| 25.3 (q) | 23 | 1.44 (s) |
| 23.6 (q) | 19 | 1.47 (br, s) |
| 22.6 (t) | 16 | 1.93 (br, m) |
| | | 1.70 (br, m) |
| 21.5 (q) | 26 | 0.89 (d, $J_{25,26} = 6.5$ Hz) |
| 19.3 (q) | 27 | 0.62 (d, $J_{25,27} = 6.5$ Hz) |
| 17.2 (q) | 20 | 1.63 (br, s) |
| | 1 | 8.50 (br, s)§ |
| | 10 | 7.77 (br, s)‖ |
| | OH on 24 | Not observed |

*Relative to $CDCl_3$ (76.9 ppm) as solvent. †Based on proton single frequency off-resonance decoupling experiments at 90 MHz (carbon-13) and proton spin-spin decoupling experiments at 100 and 360 MHz (proton). ‡$J_{gem}$ not determined. §Becomes a sharper singlet when irradiated at 6.81 ppm. ‖Becomes a sharper singlet when irradiated at 4.32 ppm.

solute configuration of **2** has been established by an x-ray crystallographic study of dihydroteleocidin B monoacetate (*14*). The similarity of the optical rotations of **1**, $[\alpha]_D$ − 171° (*c* 1.8, $CHCl_3$), and **2** $[\alpha]_D$ − 132° (*c* 0.4, $CH_3OH$) (*13*)—and the circular dichroism (CD) curves of tetrahydrolyngbyatoxin A: $[\theta]_{210\,nm}$ −22,700, $[\theta]_{223}$ −47,900, $[\theta]_{235}$ −23,700, $[\theta]_{239}$ −30,200, $[\theta]_{247}$ −25,200, $[\theta]_{265}$ −27,700, $[\theta]_{301}$ 0 (inflection), $[\theta]_{313}$ +7,100; and of dihydroteleocidin B: $[\theta]_{205\,nm}$ −15,600, $[\theta]_{225}$ −51,900, $[\theta_{234}$ −26,000, $[\theta]_{240}$ −41,500, $[\theta]_{247}$ −31,100, $[\theta]_{265}$ −33,700, $[\theta]_{300}$ 0, $[\theta]_{312}$ +7,800; in methanol indicates that the two toxins have the same stereochemistry and absolute configuration in the nine-membered ring.



**1**



**2**

At this time we do not know whether **1** is a metabolite of *L. majuscula* or a microorganism associated with the cyanophyte. Both **2** and dihydroteleocidin B have also been reported to produce intense irritation on rabbit skin (*15*), and severe irritation and eruptive vesications on human skin (*16*).

Both **1** and **2** have the same toxicities. The minimum lethal dose ($LD_{100}$) of **1** in mice is about 0.3 mg/kg by intraperitoneal injection, which is comparable to the $LD_{50}$ reported for **2** in mice, 0.22 mg/kg by intravenous injection (*14*). Tetrahydrolyngbyatoxin A and dihydroteleocidin B also have essentially the same acute toxicities as **1** and **2** (*17*).

Lyngbyatoxin A is very toxic to *Poecilia vittata* (baitfish), killing all fish within 30 minutes at a concentration in seawater of 0.15 μg/ml. Teleocidin B and dihydroteleocidin B are also highly toxic to fish and have been reported to cause the death of *Oryzias lapites* (Japanese killifish) within 1 hour at 0.01 μg/ml (*15*).

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5. The alga, which was collected from Reefer 8 pinnacle and South Elmer pinnacle at Enewetak Atoll in the Marshall Islands, was previously identified as *Lyngbya gracilis* Gomont (*4*). A reexamination of this cyanophyte shows that its morphology does not agree with the description of *L. gracilis* from Enewetak [E. Y. Dawson, *Pac. Sci.* **11**, 92 (1957)]; however, it is entirely consistent with descriptions of *L. majuscula* Gomont [E. Y. Dawson, *Pac. Sci.* **8**, 373 (1954); T. V. Desikachary, *Cyanophyta* (Indian Council of Agricultural Research, New Delhi, 1959), p. 313].
6. We have erroneously reported elsewhere [R. E. Moore, *BioScience* **27**, 797 (1977); F-J. Marner and R. E. Moore, *Phytochemistry* **17**, 553 (1978)] that *L. majuscula* from Kahala Beach, Oahu, is a variety that does not produce dermatitis.
7. A. E. Solomon and R. B. Stoughton, in preparation.
8. For the details of the gel filtration, see the experimental section for the isolation of malyngamide A from *L. majuscula* [J. H. Cardellina II *et al.*, *Phytochemistry* **17**, 2091 (1978)]. Fraction F contains the toxin.
9. Several signals in the $^1H$ NMR spectrum of the toxin are doubled in a 5:1 ratio. A temperature study at 100 MHz in [$^2H_6$]dimethyl sulfoxide at 25°, 80°, 110°, and 140°C suggests that the doubling of signals could be due to two conformations of the toxin. Considerable decomposition of toxin resulted during this experiment, and we did not determine whether the spectrum of the undecomposed toxin was still doubled. We cannot rule out the possibility that the toxin is a mixture of two isomers. The chemical shifts of the doubled signals are compatible with isomers that differ only in the position of the linalyl substituent. The chemical shifts of the larger signals (Table 1) are in agreement with the attachment of the linalyl group at C-7 as shown in **1** for lyngbyatoxin A. The chemical shifts of the smaller signals—for example, sharp doublets ($J$ = 8 Hz) at δ 7.07 and 6.99 for the two vicinal aromatic protons, a broad singlet at δ 8.75 for the indole NH, a doublet ($J$ = 18 Hz) of doublets ($J$ = 10 Hz) at δ 6.20 for the vinyl methine proton, and a singlet at δ 2.70 for the *N*-methyl group—are certainly compatible with the placement of the linalyl group at C-5. Several signals in the $^{13}C$ NMR spectrum are also doubled.
10. Two epimeric lipodipeptides, majusculamides A and B, which are major constituents of this variety of *L. majuscula*, also contain an *N*-methylvaline unit [F-J. Marner, R. E. Moore, K. Hirotsu, J. Clardy, *J. Org. Chem.* **42**, 2815 (1977)].
11. Tetrahydrolyngbyatoxin A has been fully characterized by ultraviolet, $^{13}C$ NMR, $^1H$ NMR, and mass spectral data [see J. H. Cardellina II, thesis, University of Hawaii (1979)].
12. Assignments are supported by high-resolution mass spectral data (see *11*).
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15. M. Takashima, H. Sakai, R. Mori, K. Arima, *Agr. Biol. Chem.* **26**, 669 (1962).
16. H. Nakata, H. Harada, Y. Hirata, *Tetrahedron Lett.* (1966), p. 2515.
17. At the long-term toxicity level, **1** and tetrahydrolyngbyatoxin A exhibit some activity against P-388 lymphocytic mouse leukemia. In single tests the activity or percent *T/C* of **1** was 143 (40 percent of the mice died from toxicity) and the percent *T/C* of tetrahydrolyngbyatoxin A was 152 (80 percent of the mice died from toxicity) at doses of 0.11 mg/kg. The activity is expressed as the ratio of the mean survival time of the diseased treated (T) mice (animals dying from long-term toxicity were not included) to the mean survival time of the diseased control (*C*) mice ×100.
18. This work was supported by grant CA12623-05, awarded by the National Cancer Institute, Department of Health, Education, and Welfare. We thank P. Roller for determining the high-resolution mass spectrum of **1**, M. Kashiwagi and T. R. Norton for determining the P-388 activity of **1** and tetrahydrolyngbyatoxin A, and M. McGrenra for determining the toxicity of **1** on *Poecilia vittata*. The anticancer testing was supported in part by grants from the Elsa U. Pardee Foundation, Midland, Michigan, and the Juliette M. Atherton Trust and the F. C. Atherton Trust, Honolulu. High-frequency NMR studies at the Stanford Magnetic Resonance Laboratory were made possible by NSF grant GP-23633 and NIH grant RR00711. We thank Professor H. Nakata for the sample of dihydroteleocidin B used in our circular dichroism study.

7 August 1978; revised 27 November 1978

## Ingested Mineral Fibers: Elimination in Human Urine

Abstract. *Sediment in human urine examined by transmission electron microscopy contains amphibole fibers which originate from the ingestion of drinking water contaminated with these mineral fibers. The ingestion of filtered water results in the eventual disappearance of amphibole fibers from urine. These observations provide the first direct evidence for the passage of mineral fibers through the human gastrointestinal mucosa under normal conditions of the alimentary canal.*

A key question in the evaluation of the possible human cancer risk associated with the ingestion of asbestos and other durable fibers in water, beverages, and food is whether microscopic fibers under normal conditions of the alimentary canal can migrate through the gastrointestinal mucosa (*1*). Such movement of fibers could lead to their incorporation into the bowel wall or, after hematogenous or lymphatic transport, into the peritoneum and other organ tissues. Occupational exposure to airborne asbestos dust appears to be associated with increased incidence of gastrointestinal and peritoneal cancer as well as asbestosis, bronchiogenic carcinoma, and pleural mesothelioma (*2*). Inhalation of asbestos dust is accompanied by the ingestion of many fibers cleared from the respiratory tract by mucociliary action (*3*).

The introduction of large concentrations of asbestos and other durable fibers into the pleura (*4*), peritoneum (*5*), or respiratory tract (*6*) of rodents results in malignant neoplasms. Rats fed asbestos fibers for long periods have failed to provide evidence of tumor production (*7*), but rats fed powdered chrysotile asbestos filter material (47 percent nonasbestos material included) had a significant excess incidence of malignant tumors over controls (*8*).

Some studies of tissues from animals


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